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Ariana Kendra Mercado

De La Salle University

Rafael Alfredo Batungbakal

De La Salle University

Vin Josei Gomez

De La Salle University

John Ray Estrellado

`john.ray.estrellado@dlsu.edu.ph`

De La Salle University

Aldrin Bonto

De La Salle University

André Picar

De La Salle University

Cyril John Domingo

Sultan Kudarat State University

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Influence of process variables on the green microwave-assisted low-methoxyl pectin extraction from *Calamansi* peels using citric acid

Ariana Kendra G. Mercado ¹, Rafael Alfredo G. Batungbakal ¹, Vin Josei G. Gomez ¹, John Ray C. Estrellado ^{1,2,*}, Aldrin P. Bonto ³, André E. Picar ⁴, Cyril John A. Domingo ⁵

¹Department of Science, Technology, Engineering, and Mathematics, The Academy, De La Salle University – Laguna, Laguna Boulevard, LTI Spine Road, Barangays Biñan and Malamig, Biñan City, Laguna, 4024, Philippines

² Department of Chemical Engineering, Gokongwei College of Engineering, De La Salle University, 2401 Taft Ave., Malate, Manila, 0922, Philippines

³ Department of Chemistry, College of Science, De La Salle University, 2401 Taft Ave., Malate, Manila, 0922, Philippines

⁴ School of Innovation and Sustainability, De La Salle University – Laguna, Laguna Boulevard, LTI Spine Road, Barangays Biñan and Malamig, Biñan City, Laguna, 4024, Philippines

⁵ Sultan Kudarat State University, ACCESS, EJC Montilla, City of Tacurong, Sultan Kudarat, 9800, Philippines

* Correspondence: john.ray.estrellado@dlsu.edu.ph

Abstract

The Philippines generates substantial amounts of *Calamansi* (*Citrus microcarpa*) residues from various industrial production and processing, making its peel an abundant yet underutilized agro-industrial pectin-rich waste resource. Conventional pectin extraction commonly relies on mineral acids and prolonged heating, increasing chemical and energy demands, which can be addressed using microwave-assisted extraction using a green solvent such as citric acid. This study investigated the effects and interactions of microwave and solvent treatment parameters in citric acid-assisted microwave extraction of pectin from *Calamansi* peel. Calamansi peel pectin (CPP) was extracted with varying CA concentration (5%, 10%, 15% w/v), extraction power (100 W, 200 W, 300 W), and extraction time (60 s, 120 s, 180 s) using a face-centered central composite design.

Citric acid concentration and extraction time were shown to have a statistically significant effect on the pectin yield ($p < 0.01$). The highest yield was achieved at 15% citric acid concentration, 300 W extraction power, and 180 s extraction time. Validation was conducted with three replicates, resulting in a mean yield of $27.72 \pm 4.17\%$ and a relative standard error of 12.26%, indicating a statistically reliable model. The extracted pectin was also characterized based on its equivalent weight (483.77 ± 26.37 g/mol), methoxy content ($3.95 \pm 0.82\%$), degree of esterification ($35.19 \pm 3.14\%$), and anhydrouronic acid ($57.63 \pm 8.21\%$).

Keywords

Calamansi peel pectin, microwave-assisted extraction, green solvent, citric acid, pectin extraction, response surface methodology, central composite design

1. Introduction

The extraction of pectin, a complex acidic heteropolysaccharide found in the primary cell wall and middle lamellae of higher plants, plays a particularly notable role in the overall sustainability of the pectin market [1]. The chemical and structural properties of pectin are influenced by factors such as plant source, maturity, and extraction method [2, 3]. Common pectin sources include citrus peel and apple pomace [4–6]. In line with this, agro-industrial residues such as banana (*Musa acuminata*) peels, mango (*Mangifera indica*) peels, and pomelo (*Citrus maxima*) peels have been explored as sources of pectin [7–9]. However, these commonly yield high-methoxyl pectin (HMP) that requires further de-esterification to broaden their applications [2, 10]. Hence, the exploration of alternative and locally abundant citrus as a pectin source is becoming increasingly relevant, especially as locally sourced low-methoxyl pectin (LMP) supports the green chemistry principles of “*Design for Energy Efficiency*” and “*Use of Renewable Feedstocks*” by reducing reliance on imported pectin and promoting the valorization of agro-industrial waste. Calamansi (*Citrus microcarpa*) is one of the most produced endemic citrus fruits in the Philippines, with approximately 14,000 metric tons produced from April to June 2023 [11]. Calamansi processing, however, also produces a substantial amount of underutilized biomass, with seeds, pulp, and peel making up 50-60% of the total fruit weight and commonly discarded [12]. Given its relation to commercially used citrus such as lemon and pomelo, calamansi waste has strong potential as a viable source of pectin, but remains underexplored [2, 13–15]. Pectin is also widely

used as a form of “green” packaging in food applications as a non-toxic alternative to synthetic [16, 17]. The extraction of low-methoxyl pectin (LMP) from agro-industrial waste is environmentally beneficial as it contributes to waste mitigation, resource recovery, and reduced greenhouse gas emissions, while also utilizing low-cost raw materials [18]. As a result, increasing attention is being given to sustainable pectin extraction from agro-industrial residues [19].

Given its wide use in the food, cosmetic, pharmaceutical, and packaging industries as biodegradable stabilizers, emulsifiers, thickeners, encapsulants, film-forming, and gelling agents, it is important to ensure that its extraction remains environmentally friendly [16, 20, 21]. Despite pectin’s inherent nature as a green alternative to other synthetic and harmful substances used in the aforementioned industries, its environmental implications depend on the methods through which industries extract it and the sources from which pectin is extracted. Pectin extraction involves the hydrolysis of insoluble protopectin, releasing soluble pectin into the extraction solvent [22, 23]. This process is accelerated under acidic conditions, where increased hydrogen ion concentration reduces the molecular repulsion within the polysaccharide chains, thereby promoting higher pectin aggregation and precipitation [24, 25]. Several extraction techniques have been developed to improve extraction efficiency, including conventional heat extraction, ultrasound-assisted extraction (UAE), and microwave-assisted extraction (MAE) [26]. Conventional heat extraction, the most commonly used industrial method, involves acid hydrolysis for prolonged periods ranging from 30 minutes to 6 hours [22]. However, this extended heating has been found to cause the thermal degradation and the loss of some of its functional properties [27, 28]. UAE has also been explored for pectin extraction through the use of high-frequency sound waves that disrupt cell walls, although prolonged exposure may similarly result in pectin degradation and lower yields compared to other methods [22, 26]. Given these limitations, MAE shows potential due to its substantially shorter extraction time [26]. Through using ionic conduction and dipole rotation, MAE rapidly heats the sample, dissolving the pectin into the solvent and quickly purifying the sample from pectinases, an enzyme that catalyzes the degradation of pectin [26, 29, 30]. Previous studies have demonstrated the advantages of MAE over conventional methods of extraction. For instance, MAE of pomelo peel pectin produced the highest yield while requiring only nine minutes, compared to 30 minutes for UAE and 120 minutes for conventional heating extraction [9]. Similarly, MAE of Fälticeni Apple (*Malus domestica*) pomace pectin only took a few minutes, whereas conventional heating extraction required one to

three hours [20]. These results emphasized MAE as a more efficient process capable of achieving higher yields despite taking a substantially shorter time to complete [9, 20].

The choice of extraction solvent also plays an important role. Mineral acids such as sulfuric, hydrochloric, and nitric acids often produce higher yields but can cause depolymerization and are environmentally hazardous [9, 15]. Organic acids, such as CA, on the other hand, yield high-quality pectin with low depolymerization, while being biodegradable and noncorrosive, which makes them more effective in improving pectin extraction compared to mineral acids [30, 31]. Besides this, using CA was effective in retaining the structural integrity of the produced pectin [20, 32]. When organic acids such as citric acid (CA) are used rather than mineral acids, it addresses green chemistry principles like “*Prevention*,” “*Safer Chemicals Design*,” “*Use of Safer Solvents and Auxiliaries*,” “*Use of Renewable Raw Materials*,” “*Degradation Products Design*,” and “*Accidents Prevention*,” given that CA is safer, less hazardous, biodegradable, and renewable [33]. Moreover, CA is a chelating agent, compounds whose donor atoms can simultaneously bind to a single metal ion [34]. In the context of this study, CA allows for the pectin to be more easily extracted through acidification and chelation through solubilizing the protopectin and removing metal ions from the cell wall network, which allows the pectin to more easily dissolve in the solvent [35, 36].

The yield and quality of the produced pectin are strongly influenced by several process parameters, including extraction time, power, and acid concentration [27, 37, 38]. Extended extraction at higher temperatures can increase yield, but it often reduces the molecular weight and viscosity of pectin solutions [37]. It was also found that increasing the microwave power also increases the permeation of the solvent in the cell wall matrix due to radiation, thereby increasing extraction efficiency [27]. While existing research has demonstrated that these variables can alter the physicochemical, structural, and rheological properties of the extracted pectin, their studies mostly focused on the application of the extraction technique and not the systematic evaluation of the combined effects of the parameters, especially for raw materials such as calamansi. Hence, identifying the influence of these parameters on the extraction yield of calamansi-derived pectin is crucial in exploring the parameters that enhance its extraction performance [39]. Response Surface Methodology (RSM) is a widely used approach for exploring the relationship between independent variables and response variables with the intent of exploring the combination of parameters that optimize the process [40, 41]. RSM also enables the simultaneous evaluation of

multiple process variables and their interactions using multivariate statistical analysis [41, 42]. This allows for efficient analysis by minimizing the required number of experimental runs [38]. RSM has been applied in pectin extraction studies to optimize variables such as microwave power, time, and solvent concentration, where these were systematically adjusted to determine the optimal conditions for pectin recovery [38]. Although citric acid extraction and MAE have been explored in several studies, limited research has focused on the combined effects and interactions of MAE parameters, such as solvent ratio, microwave power, and extraction duration, on pectin extraction from calamansi peel.

Thus, this study aims to determine the set of parameters that will provide the best extraction yield for microwave-assisted extraction of citric acid-extracted calamansi peel pectin. Through this approach, the main effects and interactions of citric acid concentration, extraction power, and extraction time will also be evaluated. This ensures that the green extraction process yields to a sustainable and cost-effective approach to calamansi peel pectin extraction.

2. Methodology

2.1 Materials and chemicals

The material used in this study were calamansi peels obtained from a calamansi juice processing plant in Sultan Kudarat, Philippines. Anhydrous citric acid (USP grade) used was purchased from Dalkem Corporation (Quezon City, Philippines).

The other chemical reagents include absolute ethanol (95%) from Chemline Scientific Corporation (Quezon City, Philippines), sodium hydroxide from Chem-Lab NV (Zedelgem, Belgium), hydrochloric acid from RCI Labscan Limited (Bangkok, Thailand), and phenolphthalein indicator from Loba Chemie (Maharashtra, India).

2.2 Experimental design

For the optimization of the extraction parameters, a Central Composite Design (CCD) was utilized, a common design under Response Surface Methodology (RSM). The total number of experimental runs was determined using equation 1:

$$N = 2^k + 2k + n_c \quad (1)$$

Where N is the total number of experiments, k is the number of independent variables, and n_c

is the number of replicate runs at the center point. In this study, there were three investigated factors, thus $k = 3$. The term 2^k corresponds to the factorial points, which represent the corners of the experimental design space where all factors are set at their extreme levels. The term $2k$ corresponds to the axial points, where one factor is set to its extreme while the other factors are kept at their midpoint levels, allowing for the estimation of curvature and quadratic effects. Lastly, n_c represents the center points, which are the number of replicate runs performed at the exact center of all factor levels, which are used to estimate pure experimental error and stabilize the variance.

Substituting $k = 3$ into the equation resulted in 8 factorial points, 6 axial points, and a duplicate of center points (2), giving a total of 16 experimental runs. The independent variables considered were citric acid concentration (A), microwave power (B), and extraction time (C). The selected ranges for these factors were based on and within the previously reported conditions for microwave-assisted hot acid pectin extraction. Specifically, 5 - 15% (w/v), microwave power at 100 W - 300 W, and extraction time at 60 s - 180 s [43–46]. Table 1 shows the coded and actual values for each factor. Percent yield was selected as the response variable, and all experimental runs were done in triplicate, with the results reported as the mean $\pm$ standard deviation.

Table 1. Variables and the levels for designing the central composite.

Factors	Units	Symbol	Range of Levels		
			Low (-1)	Middle (0)	High (+1)
Citric acid concentration	% (w/v)	A	5	10	15
Extraction power	W	B	100	200	300
Extraction time	s	C	60	120	180

2.3 Microwave-assisted extraction of pectin from calamansi peels

The extraction of the calamansi peel pectin was done by mixing calamansi peel powder and varying concentrations of citric acid solutions at a solute-to-solvent ratio of 1:20 [47]. The mixture

was then subjected to microwave-assisted extraction using Samsung ME711K microwave under varying microwave power as shown in Table 1. Subsequently, the extract was centrifuged at 3000 rpm for 5 minutes to separate the liquid phase. The resulting supernatant was transferred into a glass container and precipitated using 95% ethanol at a supernatant-to-ethanol volumetric ratio of 1:3 [15]. After the mixture had settled for one hour, the precipitated pectin was collected by filtration and washed twice with ethanol. Finally, the purified pectin was placed in petri dishes and freeze-dried for 24 hours at -50 °C.

2.4 Determination of percent yield

The percent yield of each run was determined through the method of other existing literature [15]. The calamansi peel powder used was weighed and represents m_1 . The final dry pectin was weighed after the extraction and represented by m_2 . The percent yield was calculated using equation 2.

$$Yield (\%) = \frac{m_2}{m_1} \cdot 100\% \quad (2)$$

2.5 Pectin characterization

2.5.1 Equivalent weight

The equivalent weight (EW) of pectin is the amount of free galacturonic acid in pectin, in which higher EW values indicate better gel-forming capabilities [48]. The extracted pectin was mixed with 5 mL of ethanol and 100 mL of distilled water. This was then followed by adding 1.0 g of sodium chloride, then six drops of phenolphthalein red indicator. Lastly, it was titrated with 0.1 N sodium hydroxide until the mixture reached a pale pink color. Equation 3 was used to compute the EW of the pectin where N and V are the normality and the volume of the sodium hydroxide used, and m_2 is the mass of the dry pectin in grams.

$$Equivalent\ weight\ \left(\frac{g}{mol}\right) = \frac{1000 \cdot m_2}{N_{NaOH} \cdot V_{NaOH}} \quad (3)$$

2.5.2 Methoxy content

Methoxy content was measured using the procedure outlined in similar research [48]. In this

method, 25 mL of 0.25 M sodium chloride solution was introduced to each solution, followed by stirring and standing at room temperature for 30 minutes. Subsequently, 25 mL of 0.25 M hydrochloric acid was added, and the mixture was titrated again with 0.1 sodium hydroxide until the pale pink color was achieved. Using equation 4, the methoxy content was calculated with m_2 as the initial mass of the pectin sample in milligrams.

$$\text{Methoxyl content (\%)} = \frac{N_{NaOH} \cdot V_{NaOH} \cdot 31}{m_2} \cdot 100\% \quad (4)$$

2.5.3 Degree of esterification

The degree of esterification (DE) was evaluated based on other existing literature [48]. The calculation followed equation 5 as shown below, where V_1 represents the volume of sodium hydroxide (NaOH) used during titration for equivalent weight determination and V_2 corresponds to the NaOH volume used in measuring the methoxy content.

$$\text{Degree of esterification (\%)} = \frac{V_2}{V_1 + V_2} \cdot 100\% \quad (5)$$

2.5.4 Anhydrouronic acid

The anhydrouronic acid (AUA) content reflects the purity level of the extracted calamansi pectin [48]. AUA was computed using equation 6, where V_1 refers to the NaOH volume used in equivalent weight determination and V_2 corresponds to the NaOH volume used in methoxy content analysis, and m_2 is the mass of the pectin sample in mg.

$$\text{Anhydrouronic acid (\%)} = \frac{176 \cdot 0.1(N_{NaOH}) \cdot (V_1 + V_2)}{1000 \cdot m_2} \cdot 100\% \quad (6)$$

2.5.6 Fourier-Transform Infrared Spectroscopy

The functional groups of the calamansi peel pectin were analyzed using Fourier-Transform Infrared Spectroscopy using the Shimadzu IRSpirit infrared spectrometer (Shimadzu Corporation, Kyoto, Japan), within 4000-500 cm^{-1} at a resolution of 4 cm^{-1} [49, 50].

216

217 2.6. Statistical analysis

218 The experimental design and response surface methodology were computed with JMP®
 219 Student Edition 19. To describe the relationship between the independent and dependent variables,
 220 the model was constructed, Shapiro-wilk test was done to determine normality, then its fitness was
 221 assessed using analysis of variance (ANOVA) and diagnostic plots. The equation below shows the
 222 second-order model used to fit the data according to the model equation 7, where Y is the response
 223 variable, β_0 is the intercept, β_i is the linear term (first-order model), β_{ii} is the quadratic term
 224 (second-order model), β_{ij} is the interaction regression coefficient, and X_i and X_j are the independent
 225 variables. The goodness of fit was evaluated by determining the coefficient (R^2) and ANOVA at a
 226 95% significance level.

$$Y = \beta_0 + \sum_{i=1}^3 \beta_i X_i + \sum_{i=1}^3 \beta_{ii} X_i^2 + \sum_{i=1}^2 \sum_{j=i+1}^3 \beta_{ij} X_i X_j \quad (7)$$

227 3. Results and Discussion

228 3.1 Evaluation of pectin yield under varying treatment parameters

229 The percent yield from each of the 16 runs was summarized in Table 2. The highest yield
 230 ($27.91 \pm 1.54\%$) was obtained at 15% citric acid, 300 W power, and a 180 s extraction time. The
 231 lowest yield ($6.91 \pm 1.73\%$) was obtained at 5% citric acid, 100 W power, and 180 s extraction
 232 time.

233 **Table 2.** Experimental matrix of pectin yield using central composite design.

Run no.	Citric Acid Concentration (%)	Extraction Power (W)	Extraction Time (s)	Yield (%)
1	5 (-1)	200 (0)	120 (0)	$7.45 \pm 1.51\%$
2	15 (1)	300 (1)	60 (-1)	$19.85 \pm 1.99\%$
3	15 (1)	100 (-1)	60 (-1)	$20.18 \pm 0.86\%$
4	5 (-1)	100 (-1)	180 (1)	$6.91 \pm 1.73\%$
5	10 (0)	100 (-1)	120 (0)	$14.89 \pm 1.75\%$

Run no.	Citric Acid Concentration (%)	Extraction Power (W)	Extraction Time (s)	Yield (%)
6	15 (1)	200 (0)	120 (0)	18.76 ± 1.49%
7	5 (-1)	100 (-1)	60 (-1)	9.10 ± 1.11%
8	10 (0)	200 (0)	180 (1)	11.26 ± 2.02%
9	5 (-1)	300 (1)	180 (1)	9.94 ± 1.35%
10	5 (-1)	300 (1)	60 (-1)	9.55 ± 1.90%
11	10 (0)	200 (0)	120 (0)	12.00 ± 1.78%
12	10 (0)	300 (1)	120 (0)	13.02 ± 5.80%
13	15 (1)	100 (-1)	180 (1)	27.06 ± 2.71%
14	10 (0)	200 (0)	120 (0)	9.52 ± 1.69%
15	15 (1)	300 (1)	180 (1)	27.91 ± 1.54%
16	10 (0)	200 (0)	60 (-1)	9.38 ± 2.55%

The Shapiro-Wilk test and homoscedasticity were conducted before performing ANOVA. The Shapiro-Wilk test was to determine if the residuals followed a normal distribution, while homoscedasticity was evaluated to determine whether the variance of residuals remained constant across observations. A *W* statistic value close to 1 and a *p*-value greater than 0.05 suggest that the data does not significantly deviate from the normal distribution [52]. From doing the test, the calculated statistic value is 0.984, while the *p*-value is 0.755. This satisfies both conditions, indicating no significant deviance from normality. In addition, a residuals versus fitted plot shown is seen to be scattered with no noticeable patterns, further suggesting homoscedasticity.

The fitness of the developed model was assessed through regression analysis and diagnostic plots, and was validated using analysis of variance. The ANOVA for the response surface model is presented in Table 3. Regression analysis resulted in a statistically significant model ($p < 0.01$). This indicates that at least one term in the model has a significant effect on the response variable [24]. Particularly, citric acid concentration (A) and extraction time (C) showed significant linear effects on the yield, while power (B) had no significant linear influence. The interaction term of citric acid (A) and time (C) showed significance with a *p*-value of less than 0.01, indicating that

their effects are dependent on each other. Significance in the quadratic terms: citric acid concentration² and power² signify a curvilinear pattern in their relationship with the yield. Overall, factors A and C, interaction term AC, and quadratic terms A² and B² were identified as significant contributors to the percent yield of the pectin extracted through MAE. The obtained R² value of 0.906984 indicated that 90.70% of the response variability can be explained by the model. Model significance is confirmed by the adjusted R-squared value of 0.884954, which is considered high.

Table 3. Analysis of variance (ANOVA) for the response surface model for pectin yield.

Source	Sum of squares	Degree of freedom (<i>df</i>)	Mean square	<i>F</i> value	<i>p</i> value
Model	1962.93	9	218.10	41.1702	<.0001
A	1504.67	1	1504.67	284.0292	<.0001
B	1.37	1	1.37	0.2589	0.6138
C	67.71	1	67.71	12.7805	0.0010
AB	3.27	1	3.27	0.6180	0.4367
AC	104.99	1	104.99	19.8180	<.0001
BC	5.28	1	5.28	0.9958	0.3246
A ²	50.69	1	50.69	9.5684	0.0037
B ²	90.44	1	90.44	17.0727	0.0002
C ²	0.51	1	0.51	0.0957	0.7587
Residual	201.31	38	5.30		
Lack of fit	28.43	5	5.69	1.0853	0.3867
Pure error	172.88	33	5.24		
SD	2.301652		R ²	0.906984	
Mean	14.17539		Adjusted R ²	0.884954	
C.V.%	16.2370%				

The generated modeling equation (equation 8) for yield was second-order and expressed using coded factors, as shown in the equation below. Positive coefficients suggest a synergistic effect. The final reduced model retained only the statistically significant terms to improve the simplicity

and accuracy of the model.

$$Y = 10.64 + 7.08A + 1.50C + 2.09AC + 2.53A^2 + 3.38B^2 \quad (8)$$

Figure 1a shows the normal probability plot, which is used to assess whether the residuals follow a normal distribution. Although there are minor deviations at the tails with a few points outside the confidence bounds, the residuals closely follow the reference line overall, indicating approximate normality [51]. Figure 1b shows the predicted and actual yields obtained from the extraction. While there are some points that fall outside the range of the confidence interval, the actual yields generally follow the predicted trend, indicating reliability [52]. The residuals versus predicted yield plot is used to assess model adequacy and to visually check the model's assumptions by revealing patterns. A random scatter of points around zero indicates a good model fit. In Figure 1c, although a few observations exhibit relatively large residuals, these are randomly distributed, with no clear systematic pattern. Therefore, indicating that the model adequately describes the relationship between the variables [53]. Lastly in Figure 1d, the residual plots fall between the green area (2) and exhibited a random distribution across the model without any observable patterns, which indicates a normal distribution of experimental errors. This confirms the relationship of the MAE parameters on pectin yield. Only two outliers exist in the graph, suggesting that some experimental errors occurred, but not to an extreme extent, as they have not crossed the red area (3.5), making the model still reliable [54].

a

b

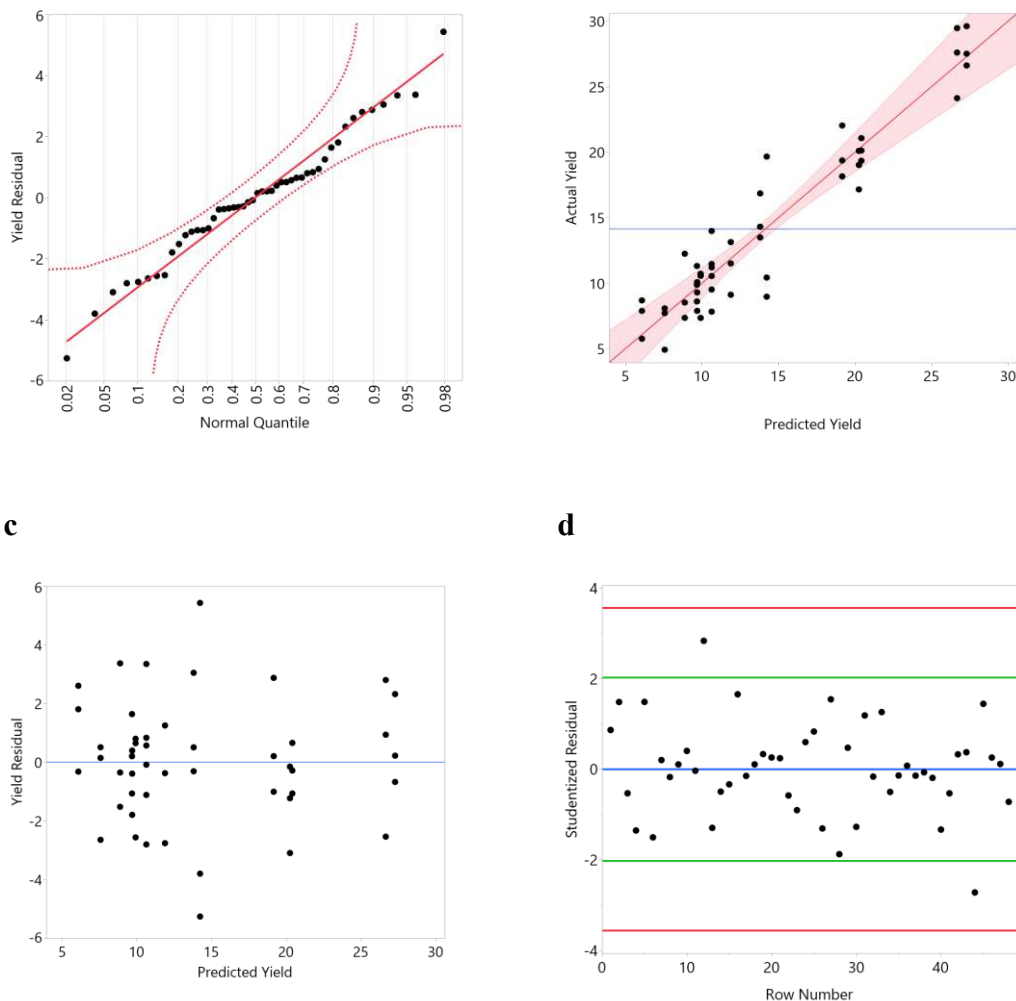


Figure 1. (a) Residual normal quantile plot, (b) actual by predicted plot, (c) residual by predicted plot, and (d) studentized residuals.

3.2 Individual effect of extraction parameters on pectin yield

Citric acid concentration was found to have a significant effect on pectin yield ($p < 0.01$), exhibiting a linear relationship. That is, citric acid concentration and pectin yield showed a directly proportional relationship. These findings are similar to literature that found that increased citric acid concentrations result in higher pectin yields from watermelon (*Citrullus lanatus*) albedo [55]. As they stated, higher concentrations of citric acid promote the breaking down and hydrolysis of protopectin into pectin due to the greater number of hydrogen ions (H^+).

On the other hand, extraction power had no significant effect on the calamansi peel pectin yield ($p > 0.01$). From extraction powers of 100 W to 300 W, 200 W had the lowest yield. Despite

having higher power than 100 W, 200 W compromises pectin structure due to degradation caused by the higher heat, leading to lower pectin yields. 300 W, despite being a higher power, outpaces the degradation process of the pectin, retaining the pectin yield, though not considerably higher than 100 W [56].

Extraction time has a statistically significant main effect on yield ($p < 0.01$), indicating that changes in the extraction time influence the yield, although to a lesser extent than changes in citric acid concentration. The main effects plot for extraction time shows a positive slope, indicating that yield increases with increasing extraction time. These findings are consistent with previous studies on MAE, such as the extraction of pectin from dragon fruit peels and tangerine [19, 43]. However, other studies on black carrot pomace and lemon peel powder reported that prolonged extraction times can reduce yield due to overexposure to microwave energy, leading to pectin diffusion or degradation of pectin chains [15, 57].

3.4 Interactive effects between independent variables on pectin yield

The three-dimensional (3D) surface plots in Figure 2 illustrate the combined effects of the three variables on the percent yield of pectin, namely: (A) citric acid concentration, (B) extraction power, and (C) extraction time. The plots present the pairwise interactions between variables, with Figure 2a showing the interactive effects of citric acid concentration (A) and extraction power (B), Figure 2b presenting the interaction between citric acid concentration (A) and extraction time (C), while Figure 2c depicting the combined influence of extraction power (B) and extraction time (C) on the percent yield.

The diagnostics of the model statistical properties is shown in Figure 1. It includes the residual normal quantile plot, actual by predicted yield, residual by predicted yield, and studentized residuals plot.

4.2.1 Interactive effect of citric acid concentration (A) and extraction power (B)

The interaction between citric acid concentration and extraction power was not statistically significant ($p > 0.01$). As shown in Figure 2, pectin yield increases when both citric acid concentration and extraction power increase. However, ANOVA determined that there is no significant interaction between the two, indicating that these trends are from additive factors and not synergistic effects. These contribute to pectin extraction in different ways. Increased power

results in the production of heat energy from polar water, which consequently results in cell wall rupturing, increasing the yield [9]. Citric acid concentration increases pectin solubility by breaking down pectin chains through the disruption of ionic bonds. Divalent cations are bound, allowing pectin to dissolve more easily into the solvent [58]. These two parameters are not interdependent as they affect yield in two distinct ways; power affects cell wall rupturing, while citric acid affects pectin chain degradation.

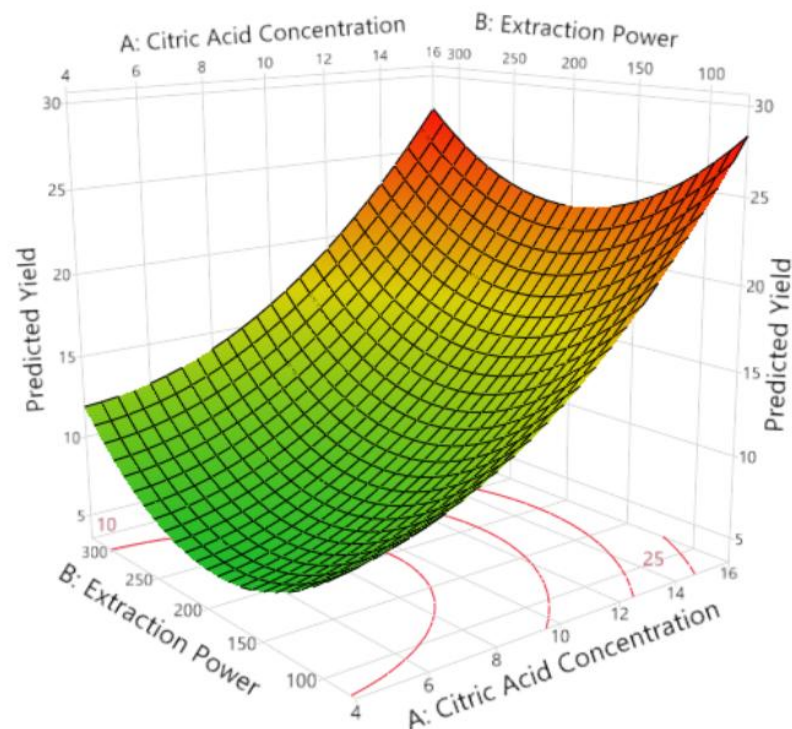


Figure 2. Response surface plot for the interaction between citric acid concentration (A) and extraction power (B).

4.2.2 Interactive effect of citric acid concentration (A) and extraction time (C)

The interaction between citric acid concentration (Factor A) and extraction time (Factor C), as shown in Figure 2b, was highly significant ($p < 0.01$), indicating that the effect of each factor on yield depends on the level of the other. As both Factor A and Factor C increased, the yield also increased, suggesting a synergistic effect. This trend is clearly reflected in the 3D surface plot, where the highest yield was observed at the maximum levels of both factors. These results suggest that optimal yield can be achieved by simultaneously increasing citric acid concentration and

extraction time, consistent with previous studies on MAE of dragon fruit pectin and MAE of lemon peel powder pectin [15, 43]. This is because higher citric acid concentrations increase the availability of protons, which hydrate carboxyl groups in the pectin, that lead to reduced repulsive forces and promotes precipitation [55]. This is then further assisted by the extended extraction time, as this prolongs the interaction between the citric acid and the calamansi peel pectin.

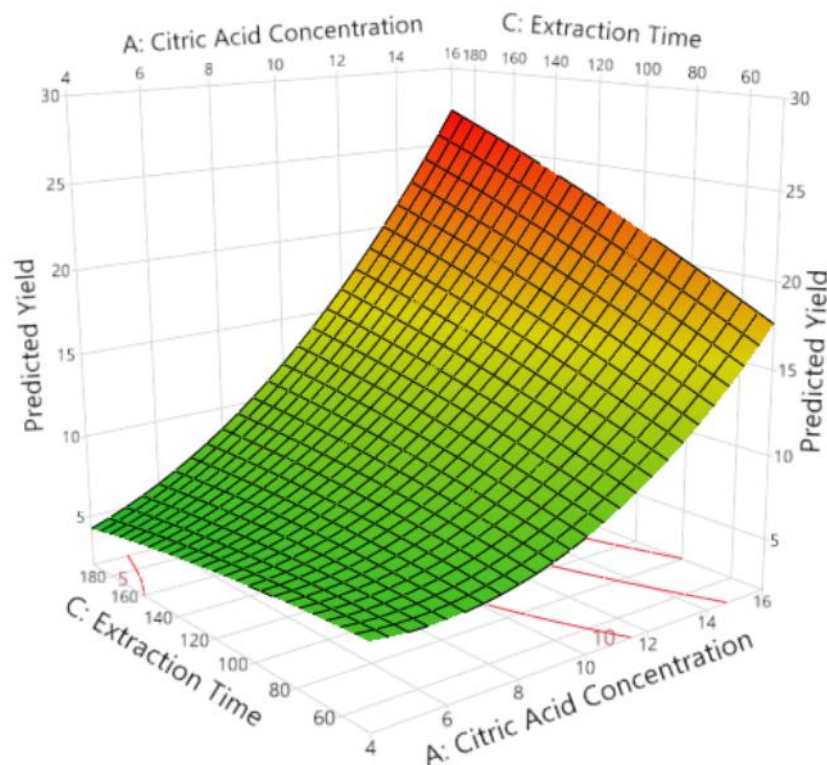


Figure 3. Response surface plot for the interaction between citric acid concentration (A) and extraction time (C).

4.2.3 Interactive effect of extraction power (B) and extraction time (C)

The interaction between extraction power and extraction time held no significant relationship ($p > 0.01$), indicating that the two factors do not rely much on each other. As observed in Figure 2c, there is a noticeable decrease in yield at 200 W extraction power, while 100 W and 300 W have almost identical yields, with 300 W being slightly higher. While increased extraction times indeed led to higher yields, there was no interactive effect with varying extraction powers, and the figure

shows that the two factors are additive rather than synergistic. This is because extraction time focuses on lengthening the exposure of the pectin sample to the extraction, rather than actively penetrating cell walls and cause cell rupture to release the pectin [59].

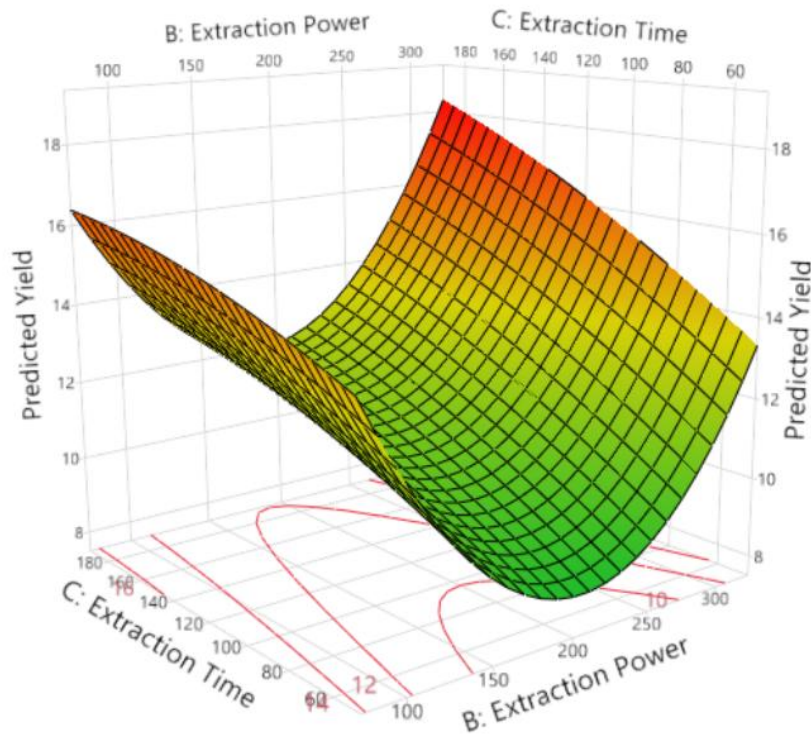


Figure 4. Response surface plot for the interaction between extraction power (B) and extraction time (C).

3.5 Model validation for highest pectin yield

The highest pectin yield was determined at a citric acid concentration of 15%, extraction power of 300 W, and extraction time of 180 s. Under these conditions, the predicted response was determined to be 27.29%, based on the prediction profiler shown in Figure 3.

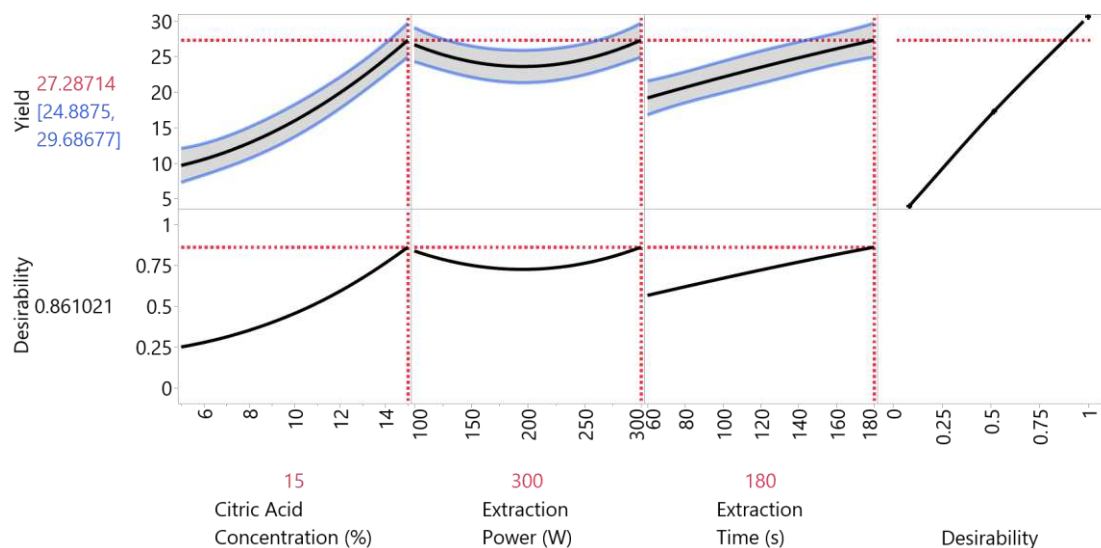


Figure 3. Prediction profiler generated in JMP showing the effects of citric acid concentration (%), extraction power (W), and extraction time (s) on pectin yield and overall desirability. Optimal conditions (15%, 300 W, and 180 s) maximize desirability. Blue values indicate the predicted ranges, the red predicts the mean, and the shaded regions represent the confidence intervals [60].

The agreement between predicted and observed values was evaluated using relative standard error (RSE), where values below 25% are considered statistically reliable, while those exceeding 25% were considered to have high sampling error [61]. The percent yields for the replicates are 30.69%, 29.55%, and 22.92%, respectively and have a mean value of $27.72 \pm 4.17\%$ with an RSE of 12.26%, which is below the acceptable 25% threshold.

3.6 Characterization of pectin extracted under the highest yield

The pectin samples extracted under 15% citric acid concentration, 300 W power, and 180 s were characterized based on equivalent weight, methoxy content, degree of esterification, and anhydrouronic acid content.

The pectin's equivalent weight was 483.77 ± 26.37 g/mol, which is higher than recorded equivalent weight in existing studies that researched various citrus fruit pectins (248.21 to 387.43 g/mol). Higher EW indicates an increased preservation of the pectin backbone as it is associated with fewer free acid groups [30]. This shows that the optimal conditions used for microwave-assisted extraction helped reduce the degradation of the calamansi pectin. This result may also be

attributed to the weak acid nature of citric acid, which, despite its relatively higher concentration (15%), is still able to preserve the structural integrity of the pectin [9].

The methoxy content recorded was $3.95 \pm 0.82\%$, and it was slightly lower than the recorded values compared to similar research characterizing citrus pectin at 4.26-6.09% [30]. This falls within the 2.5–7.12% range, classifying the sample as low-methoxyl pectin [48]. Higher citric acid concentrations better facilitate de-esterification in the pectin [62]. When combined with the increased thermal energy due to the higher extraction power, the de-esterification process is sped up, which lowers the methoxyl content, making the pectin low-methoxyl.

The obtained pectin is also classified as low-methoxyl pectin, with a DE value of $35.19 \pm 3.14\%$. Similar to what was previously stated from Sarah *et al.* [62], when a higher citric acid concentration and extraction power is combined, the process of de-esterification is sped up which also decreases esterified groups in the pectin, overall decreasing the degree of esterification. The AUA recorded ($57.63 \pm 8.21\%$) were lower than the recommended value for food and pharmaceutical use at 65% [63]. The lower AUA value exhibited by the pectin indicate the presence of impurities or the incomplete extraction of the pectin and is mainly due to the use of citric acid. While the increased extraction time and power increases AUA values, the use of citric acid is not enough to bring the AUA value higher than 65% due to it being a weaker acid. It is unable to provide effective proton activity, unlike strong mineral acids [64]. This suggests that additional steps may be necessary to improve the quality of the pectin, but overall, the conditions used were effective in pectin extraction.

Extracted calamansi peel pectin was compared with commercial citrus pectin from Dalkem Corporation as a baseline in terms of FTIR spectroscopy, as seen in Figure 4. The FTIR analysis of orange pectin served as a benchmark for pectin polysaccharide signals and their corresponding functional group, as indicated by the vertical dashed lines [65]. The broad band at 3336 cm^{-1} represents the O-H stretching, which is a result of the inter- and intramolecular hydrogen bonding of the sample [66]. The C-H stretch at 2929 cm^{-1} corresponds to the vibrations of CH, CH₂, and CH₃, which are more pronounced in the CPP as opposed to the baseline. The peaks along 1737 cm^{-1} and 1640 cm^{-1} are ascribed to the esterified carboxyl groups and the non-esterified carboxyl groups bands of the pectin structure [67]. In the fingerprint region, the peak at 1080 cm^{-1} is indicative of the presence of both C-O-C and C-O-H stretching vibrations, which identify the

glycosidic linkages and pyranose ring vibrations of the backbone structure of the pectin [68]. Although the spectra of both pectin samples are highly similar in terms of their characteristic functional groups, the deeper bands of the CPP spectra indicate strong signal absorption as a result of the purity of the sample. This confirms the extracted CPP pectin nature of the sample, as well as the abundance of its distinctive functional groups.

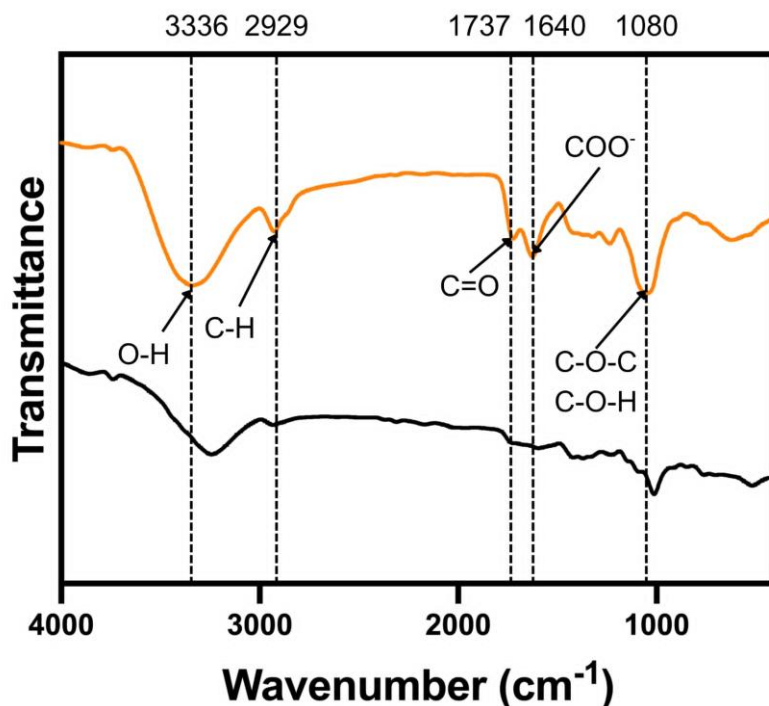


Figure 4. FTIR spectra of extracted calamansi peel pectin (orange, top) and commercial citrus pectin (black, bottom), with markers for pectin polysaccharide signals (vertical dashed lines) based on FTIR analysis.

5. Conclusions

After successfully extracting pectin from calamansi peels using citric acid MAE, the optimal combination of parameters was shown to be 15% CA concentration, 300 W extraction power, and 180 s extraction time. This combination achieved the highest yield: $27.91 \pm 1.54\%$. On the other hand, the lowest yield was obtained at 5% CA concentration, 100 W power, and 180 s extraction time: $6.91 \pm 1.73\%$. The synergistic relationship identified was between CA concentration and extraction time, which showed a positive linear effect on the yield. The overall model has been

shown to be reliable, can explain 90.70% of the response variability, and is significant with a high adjusted- R^2 value of 0.88, ultimately showing how the model can predict pectin yield based on the discussed parameters. The results of the optimization were consistent with validation trials, all of which showed relative standard errors from the predicted yield below 25%. The effects of the parameters aligned with other existing literature. The pectin extracted under optimal conditions was characterized as low-methoxyl pectin, which shows potential in food applications as a gelling agent in low-sugar jams and jellies, as well as pharmaceutical applications as an excipient in drug formulation due to its stabilizing properties. Recommendations include improving quality, improving purity, scaling the process for mass production, conducting the study with other raw materials, and evaluating the applicability of the extracted pectin for industry uses. In summary, this study was able to provide new information on the citric acid microwave-assisted extraction of calamansi peel pectin, highlighting a sustainable alternative to conventional pectin extraction methods.

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Author contributions

A. K. G. M.: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Visualization, Writing - Original Draft; R. A. G. B.: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Visualization, Writing - Original Draft; V. J. G. G.: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Visualization, Writing - Original Draft; J. R. C. E.: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Project administration, Resources, Supervision, Validation, Visualization, Writing - Original Draft, Writing - Review & Editing; A. E. P.: Investigation, Methodology, Visualization, Writing - Original Draft; C. J. A. D.: Resources, Validation, Writing - Review & Editing; A. P. B.: Conceptualization, Investigation, Supervision, Validation, Writing - Review & Editing;

456

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461 **Data availability**

462 Data are provided within the manuscript.

463

464 **Declarations**

465 *Ethics approval and consent to participate*

466 The calamansi peel used in the study were sourced as food-processing by-products from a local
467 calamansi plant in Sultan Kudarat, Philippines, through Sultan Kudarat State University. No
468 collection of living plants or plant tissues from natural populations was done; therefore, no
469 collection permit or special license was required for the plant material used in this study.

470 *Consent for publication*

471 Not applicable.

472 *Competing interests*

473 The authors declare no competing interests.

474

475 **8. References**

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