Research Article

Received: 16 December 2019 Revised: 11 June 2020 Accepted article published: 21 June 2020 Published online in Wiley Online Library:

(wileyonlinelibrary.com) DOI 10.1002/jsfa.10600

Optimization of pectinase-assisted extraction of *Annona muricata* L. juice and the effect of liquefaction on its pectin structure

Calister Wingang Makebe,[a,b] Zangue Steve Carly Desobgo,[c] Wilson Agwanande Ambindei,[a] Abraham Billu,[b] Emmanuel Jong Nso[a] and P Nisha[b*]

Abstract

BACKGROUND: Soursop (*Annona muricata* L.) is an underutilized tropical and subtropical fruit with high nutritional and therapeutic benefits. This fruit is faced with enormous post-harvest losses due to its high perishability. This work was aimed to optimize the pectinase-assisted extraction conditions of soursop juice using Doehlert design and to study the effect of pectinase on its pectin structure.

RESULTS: The predicted models were validated for all the responses studied and the regression coefficients ranged from 0.905 to 0.987 ($P \leq 0.05$). An incubation time of 172 min, enzyme concentration of 0.04% (*w/w*) and incubation temperature at 42.9 °C were found to be the optimal conditions for soursop juice extraction, which resulted in 75.20%, 3.74, 7.35 °Brix, 87.06%T, and 0.44% MAE for soursop juice yield (%), pH, total soluble solids (TSS) (°Brix), clarity (%T) and titratable acidity (% malic acid equivalent, MAE), respectively. Morphologically, untreated soursop pulp presented a non-uniform spherical surface; enzyme hydrolyzed soursop exhibited ruptured and wrinkled surface; meanwhile for the different pectin obtained, untreated soursop pectin depicted porous surface and enzyme hydrolyzed soursop pectin showed whirling rough surface. Fourier-transform infrared (FTIR) confirmed the presence of similar chemical group stretching and vibrations in commercial pectin and soursop pectin.

CONCLUSION: Under the optimum conditions, the numerical predictions were similar to the experimental data obtained, thus confirming the validity of the models. Application of enzyme treatment caused the breakdown of pectin structure as illustrated by scanning electron microscopy (SEM) and FTIR analyses.

Supporting information may be found in the online version of this article.

Keywords: *Annona muricata* L.; juice; pectinase; optimization; liquefaction; pectin

1 INTRODUCTION

Annona muricata L., commonly called soursop, is a tasty fruit widely grown in the tropical and subtropical regions of the world, including South America, Africa, Asia, and Australia.[1,2]

As a result of the increasing information on the high nutritional profile and content of health-protective phytochemicals as well as the peculiar flavor of this exotic fruit, it has received considerable research attention during the last decade.[3] The fruit is reported to be a good source of fibers, minerals [phosphorus (P), iron (Fe), and calcium (Ca)], vitamins (B1, B2, B3, and C) and amino acids [methionine (Met), lysine (Lys) and tryptophan (Trp)].[4] Previous findings revealed that *A. muricata* L. is a rich source of bioactive phenolic compounds with antidiabetic and antihypertensive potentials[5] and anticancerogenic properties.[6] Antioxidant activity and inhibition against α-amylase, α-glucosidase, and angiotensin-I converting enzyme (ACE) of *A. muricata* L. pericarp extract was also reported.[5] Unfortunately, the highly perishable nature of soursop fruit aggravated by the lack of appropriate post-harvest techniques leads to its post-harvest losses at about 76% of its total production.[4] Towards curbing post-harvest losses, several processing techniques such as spray drying of soursop pulp,[7] and processing into products such as puree,[8,9] nectar,[10] jam,[11] and juice[12–14] have been reported.

Soursop pulp is a suitable feedstock for juice production. However, the processing of this fruit pulp into juice is difficult due to its pectinaceous nature, which prevents the diffusion of solutes

* *Correspondence to: P Nisha, CSIR – National Institute for Interdisciplinary Science and Technology (NIIST), 695019, Trivandrum, Kerala, India. E-mail: bp.nisha@yahoo.com*

a *Ecole Nationale Supérieure des Sciences Agro-Industrielles (ENSAI), Université de Ngaoundéré, Ngaoundéré, Cameroon*

b *CSIR – National Institute for Interdisciplinary Science and Technology (NIIST), Trivandrum, India*

c *Department of Food Processing and Quality Control, University Institute of Technology (UIT), The University of Ngaoundere, Ngaoundere, Cameroon*

during the extraction process.[9,15] Previous studies reported that depectinization improves the yield of extraction via increase liquefaction, reducing viscosity,[16] higher release of phenolic and nutritional components,[17] reduce efforts to press,[9] enhances clarification[17] and increases quality[16] of several fruit juices such as banana,[16,18] guava[19] and mango.[20] The use of enzymes for the extraction of soursop has been reported earlier, and the enzyme-treated soursop could be used as juice,[15] puree,[9] and spray-dried powder.[21] Chang *et al.*[9] studied the impact of enzyme concentration (pectinase, α-amylase, and cellulase) and incubation time on soursop puree extraction. In a study carried out by Yusof and Ibrahim,[15] the authors evaluated the quality of soursop juice after treatment with varying concentrations of pectinase and for different time intervals at a fixed temperature. The optimum conditions of treatment were found to be 0.075% of pectinase for an incubation time of 2 h, which improved the juice yield by 41%. However, the earlier studies did not consider the impact of liquefaction temperature on the extraction yield and the quality of soursop juice. As extraction temperature is an important parameter with respect to enzyme activity and the extraction yield, it is important to optimize the extraction conditions with respect to enzyme concentration, incubation time and temperature.

Response surface methodology (RSM) is a modeling technique used in the analysis of scientific problems by grouping mathematical and statistical systems, wherein a given outcome/response is impacted by many variables.[22] RSM is employed to optimize the outcome/response by adopting an appropriate experimental design. Optimization is the improvement of a process efficiency so as to maximize the outcome.[22] In 1970, Doehlert proposed a plan with a uniform distribution of the experimental points in the experimental space.[23] Compared to the other experimental designs, this design is practical, economical, more uniform, has few experimental application points and higher efficiency.[16] With this background, this study aimed to understand the interactions between the extractions conditions of soursop juice, namely enzyme concentration, incubation time, and temperature, using pectinase-assisted extraction, and to determine the optimal extraction conditions using the Doehlert design (DD). The study also aims to evaluate the effect of liquefaction on its pectin structure.

2 MATERIALS AND METHODS

2.1 Raw material

Fully fresh mature soursop fruits, yellowish-green in color were purchased directly from a farmer in Penja, Littoral region of Cameroon. The fruits were washed under a running tap and then immersed in 2% hypochlorous acid, drained and allowed to ripen at ambient temperature (25 ± 2 °C) for 72 h. After ripening, the fruits were peeled, seeds were removed and then crushed (to increase the surface area for the action of pectinase) intermittently at an interval of 2.5 min for 5 min using an electric mixer (MG 218; Zodiac Preethi, Chennai, India). The crushed soursop pulp was stored in sealed plastic bags at −20 °C until use.

2.2 Enzyme and chemical reagents

Pectinase from *Aspergillus niger* with the enzymatic activity of 1.11 unit/mg (pH 4.0 and 50 °C) was purchased from Sigma-Aldrich, Vandtårnsvej 62A, 2860 Søborg, Denmark.

Sodium hydroxide, Folin Ciocalteu's phenol reagent, sodium carbonate, and sulfuric acid were purchased from Himedia, Bengaluru, India. Carbazole, D-(+)-galacturonic acid monohydrate, commercial citrus pectin, gallic acid standard, sodium potassium tartrate, potassium ferrocyanide, and zinc acetate were purchased from Sigma-Aldrich, Steinheim, Germany. All chemicals were of analytical grade.

2.3 Soursop juice extraction

The crushed soursop pulp was thawed at 4 °C for 12 h prior to extraction. The crushed soursop pulp (150 g) was weighed into a beaker, followed by the addition of water to obtain a final water-to-substrate ratio of 1:1 *v/w*. It was placed in a temperature-controlled water bath at the appropriate temperature then pectinase was added to give a final enzyme-to-substrate ratio, as mentioned for each trial of the DD. Hydrolysis was carried out with continuous shaking at an interval of 15 min using a Remi Motor agitator (RQ 122; Elektrotechnik Ltd, Kolkata, India).

After liquefaction, the pectinase was inactivated by heating the sample at 95 °C in a water bath for 5 min. Sample was cooled at room temperature and centrifuged at 6000 × *g* for 15 min at 25 °C using a centrifuge (7780; Kubota, Bunkyo-ku, Tokyo, Japan). The juice extract (supernatant) was carefully separated from the pellet and used for further analysis.

2.4 Modeling and optimization

The DD was used as an RSM to model the factors and obtain optimal conditions for the extraction of soursop juice. Based on literature,[19,24–26] preliminary studies were carried out to define the experimental domain of independent variables, which were as follows: incubation time (30–180 min), enzyme concentration (0.01–0.1%), and incubation temperature (35–55 °C). The independent variables were studied at three levels (−1, 0, +1), with a total of 17 trials with five center points. The dependent variables (responses) were: yield (%), pH, total soluble solids (TSS) (°Brix), clarity (%T) and titratable acidity (% malic acid equivalent, MAE). Once the optimal conditions were obtained using DD, soursop juice extraction was carried out under these conditions to validate the accuracy of the model, and the extract was further analyzed.

For predicting the optimal point, a mathematical model was fitted to correlate the independent variables and the dependent variables. The mathematical model employed was a second-degree polynomial model with linear, quadratic, and interaction terms, as represented in Eqn (1).

$$Y_i = \beta_0 + \sum \beta_i x_i + \sum \beta_{ii} x_i^2 + \sum \beta_{ij} x_i x_j \quad (1)$$

where, Y_i is the response, X_i and X_j are the variables, β_0 is a constant, β_i is the coefficient of the linear term (β_1, β_2, β_3), β_{ii} is the coefficient of the quadratic terms (β_{11}, β_{22}, β_{33}) and β_{ij} is the coefficient of the interaction term (β_{12}, β_{13}, β_{23}).

To this effect, the mathematical model equations were analyzed by ANOVA (analysis of variance) using Minitab 19.1.1 (2019 Minitab, LLC, State College, PA, USA) software; meanwhile, response surface curves were obtained using OriginPro 9.0.0 (OriginLab Corporation, Northampton, MA, USA), to describe the individual and interactive effects of the factors on the responses.

Mathematical model validation is valuable for the prediction of responses in a given domain studied; hence, in the present study, the experimental and theoretical values given by the models were compared. Furthermore, the linear regression coefficient, absolute average deviation (AAD), bias factor (B_f), and exactitude factor (A_f) were used to validate the models.[27]

2.5 Physicochemical analysis

Moisture, crude fiber, crude fat, crude protein, ash and carbohydrate contents of the crushed soursop pulp were determined by the official methods of the Association of Official Analytical Chemists (AOAC).[28]

2.5.1 Yield

The soursop juice yield (% *v/w*) was evaluated as a percentage of the volume of juice extracted (supernatant) and obtained after centrifugation from the initial mass of the pulp, as presented in Eqn (2):

$$\text{Yield}\,(\%v/w) = \frac{\text{Volume of extract (supernatant)}}{\text{mass of pulp}} \times 100 \qquad (2)$$

2.5.2 pH

The pH value of soursop juice was read using a pH-meter (pH 700; Eutech, Ayer Rajah Cresent, Singapore). The pH-meter was calibrated as stipulated in the pH-meter operating manual by dipping the electrode for a specified time in the neutral buffer (7.01), acid buffer (4.01) and basic buffer (10.01).

2.5.3 Total soluble solids (TSS)

The total soluble solids (TSS) content of soursop juice was obtained using a hand refractometer (0–32% Brix; ERMA, Nashik, Maharashtra, India**)**. The refractometer was calibrated using distilled water, and °Brix of soursop juice was then measured.

2.5.4 Titratable acidity

Titratable acidity was measured according to the standard method.[28] To 10 mL of soursop juice aliquot was added a few drops of phenolphthalein indicator then titrated against standardized 0.1 N sodium hydroxide to a pink color change which persisted for at least 30 s. The results were expressed in percentage of malic acid equivalent (%MAE).

2.5.5 Clarity

Clarity of the soursop juice was measured at 660 nm by noting the percentage transmittance (%T) using a spectrophotometer (UV-2600; Shimadzu, Kyoto, Japan) according to Sagu *et al.*[16]

2.6 Pectin analysis

2.6.1 Galacturonic acid determination

Pectin quantification in terms of galacturonic acid content in crushed soursop pulp before and after enzymatic hydrolysis was obtained using the method described by Ninga *et al.*[19] with slight modifications. Sample (1 g) was extracted with 0.05 N sodium hydroxide for 30 min. Then, 6 mL of concentrated sulfuric acid was added to the above extract. The mixture was homogenized and heated at 95 °C for 10 min in a water bath and then cooled in an ice bath. The mixture was further added with 0.5 mL of 0.15% carbazole and incubated for 15 min at 25 °C to observe a color change. Absorbance was read at 520 nm using a spectrophotometer (UV-2600; Shimadzu). After enzymatic action, pure (95.6%) ethanol was added to the hydrolyzed soursop at the ratio of 1:3 (*w/w*) while agitating, and the mixture was kept at 4 °C overnight, followed by centrifugation at 10 000 × *g* for 15 min at 4 °C, which was aimed at precipitating the pectin. The supernatant was discarded, and the pellet was washed successively with 50%, 75%, and pure (95.6%) ethanol to eliminate any monosaccharides and disaccharides present in the pellet before further analysis. The value of galacturonic acid was determined and expressed as gram per kilogram of sample.

2.6.2 Pectin extraction

Pectin was extracted as described by Ranganna[29] with some modifications. Soursop sample was diluted in distilled water, and the pH was reduced to 2.0 using 0.5 mol/L hydrochloric acid and was allowed to boil for 1 h at 90 °C while stirring every 15 min. Sample was rapidly filtered using a muslin cloth, and absolute ethanol was added to the liquid phase at the ratio of 1:3 (*v/v*) and centrifuged at 5000 × *g* for 10 min at 4 °C. The pellet was rinsed with acetone to remove impurities and then freeze-dried.

2.6.3 Equivalent weight, methoxyl content, total anhydrouronic acid content and degree of esterification

After soursop pulp pectin extraction, the pectin was tested for the absence of amide as described by Ranganna.[29] The equivalent weight analysis was carried out by mixing 0.5 g of the pectin with 5 mL of ethanol, sodium chloride (1 g), and 100 mL of distilled water. Complete dissolution of the pectin was ensured by stirring before the addition of six drops of red phenol indicator then titration with standardized 0.1 N sodium hydroxide. To the neutral solution of equivalent weight, 25 mL of 0.25 N sodium hydroxide was added, shaken thoroughly, and kept at ambient temperature (25 ± 2 °C) for 30 min. To this, 25 mL of 0.25 N hydrochloric acid was added then titrated to purple color. The anhydrouronic acid content and the degree of esterification were calculated using the formula developed by Ranganna[29] and expressed in percent.

2.7 Scanning electron microscopy (SEM)

A scanning electron microscope (EVO 18; Zeiss, Oberkochen, Germany) was used to observe the morphological change of the treated sample. Untreated soursop, hydrolyzed soursop residue, soursop pectin, and hydrolyzed soursop pectin were lyophilized, cut/spread on the metallic plate and, coated with a thin layer of gold for an hour. Micrographs were obtained at a magnification of 20 000× with 15 kV of acceleration.

2.8 Fourier-transform infrared (FTIR) spectroscopy

The Fourier-transform infrared (FTIR) spectra of commercial pectin, pectin from untreated soursop, and hydrolyzed soursop were analyzed using an attenuated total reflectance-Fourier-transform infrared (ATR-FTIR) spectrometer (IR spectrophotometer; Bruker Optik Gmbh, Ettlingen, Germany). The sample was deposited on the surface of a diamond crystal and pressed with a system press tip flap. Spectra were registered in transmission mode within the wavenumber range of 4000–500 cm^{-1} with 32 scans per spectrum at a resolution of 8 cm^{-1}.

3 RESULTS AND DISCUSSION

3.1 Proximate composition and physicochemical properties of soursop

The proximate composition and physicochemical properties of soursop are presented in Table 1 alongside the values reported in the literature. The soursop fruit investigated in the current study has higher crude fat value than that reported by Sanusi and Bakar.[3] Similarly, the values of ash and crude fiber were higher than those pointed out by Ndife *et al.*[14] Meanwhile, the carbohydrate, protein, and moisture contents showed similarities to the findings of Badrie and Schauss,[4] Ndife *et al.*[14] and Pinto *et al.*[30] Some researchers termed this fruit to be a high caloric

Table 1 Proximate composition and physicochemical properties of soursop pulp

Parameter	Values	Literature values
Moisture content (g/kg)	822.7 ± 5.10	82.8 g/100 g[4]
Ash content (g/kg)	24.4 ± 0.01	1.83 ± 0.15%[14]
Crude fat content (g/kg)	15.0 ± 0.01	0.74–0.97 ± 0.06%[4,12,14]
Crude fiber content (g/kg)	39.96 ± 0.01	2.26 ± 0.10%[14]
Carbohydrate content (g/kg)	173.3 ± 9.40	17.25 ± 0.1 g/100 g[30]
Protein content (g/kg)	50.8 ± 2.50	5.35 ± 0.24%[14]
pH	3.66 ± 0.010	3.68 ± 0.16[9]
Color		
*L**	82.08 ± 0.005	74.44 ± 0.60[9]
*a**	0.09 ± 0.005	−0.94 ± 0.16[9]
*b**	16.64 ± 0.005	10.19 ± 0.27[9]
Titratable acidity (% MAE)	4.13 ± 0.630	1.50 ± 0.05 (%)[14]
Reducing sugars (g/kg)	131.1 ± 3.00	9.91 ± 0.04 (g/100 g)[31]
Total polyphenol content (g GAE/kg)	0.8 ± 0.040	34.63 ± 1.21(μg/100 g)[13]
Total flavonoid content (g quercetin/kg)	0.01 ± 0.000	NA
Total soluble solids (°Brix)	11.12 ± 0.300	11.0 ± 0.4[8]

MAE, malic acid equivalent; GAE, gallic acid equivalent; NA, not available.

value fruit because of the high carbohydrate content.[30] These discrepancies in the proximate composition of soursop pulp compared to other studies could be due to the dissimilarities in geographical conditions, agronomical practices, fruit variety and maturity stage.

The physicochemical properties of the soursop pulp revealed that the values of pH and TSS were similar to those reported in other works.[8,21] Nevertheless, higher values were obtained in the present study for color, reducing sugars, titratable acidity and total polyphenol content compared to those previously reported in similar studies (shown in Table 1).[9,13,14,31] The variation in the physicochemical properties of the soursop pulp is attributed to fruit varietal differences, horticultural practices, and climatic conditions,[30] meanwhile other findings associated these discrepancies to the ripening processing typical of *Annonaceae* fruits due to the decomposition of complex carbohydrates and organic acids.[31]

3.2 Pectin analysis

Pectin yield, equivalent weight, methoxyl content, anhydrouronic acid content, degree of esterification, and galacturonic acid values are represented in Table 2. The pectin yield is in accordance with other findings, which proposed that the pectin in soursop could be applied as an important by-product.[30] The equivalent weight represents an index of pectin's jelly-forming potential under suitable conditions. Soursop pectin was of the low methoxyl type since the value of degree of esterification was below 50%. Hence, it exhibited a very slow gel set and the possible formation of thermo-reversible gels at low pH and calcium ions. However, soursop pectin has the capacity to form gel even in the presence of lower sugar concentration. The degree of esterification obtained in this study was similar to that reported by Liew *et al.*,[32] who obtained a degree of esterification of 41.67% for passion fruit peel pectin extracted at pH 3.3 for 120 min. The anhydrouronic acid content of pectin was below 65%, which showed that it contained some impurities like proteins, starches, and sugars. This anhydrouronic acid result is in agreement with those obtained from banana peel pectin that displayed an anhydrouronic acid content ranging from 55.61 to 58.77%.[33]

Table 2 Soursop pectin extraction and characterization

Parameter	Values
Yield (%)	9.60 ± 0.001
Galacturonic acid (g/kg)	5.96 ± 0.370
Galacturonic acid after hydrolysis (g/kg)	15.42 ± 0.978
Amide test	Negative
Equivalent weight	559.67 ± 17.240
Methoxyl content (%)	2.86 ± 0.540
Total anhydrouronic acid content (%)	48.0 ± 1.310
Degree of esterification (%)	33.95 ± 4.320

3.3 Statistical analysis and model fitting of DD

Incubation time, enzyme concentration, and incubation temperature were computed for the optimization of yield, pH, clarity, TSS, and titratable acidity by the use of a DD. The experimental matrix of the DD (both coded and real values) and the responses are presented in Table 3 meanwhile, the coefficient of models (Y_1, Y_2, Y_3, Y_4, and Y_5) and their respective *P*-values are shown in Table 4. The coefficients of models were validated based on the *P*-values, with *P*-values less than or equal to 0.05. However, the mathematical models in this study were valid upon consideration of R^2, adjusted R^2, AAD, A_f and B_f.[27] A model was claimed workable if the R^2 was greater than or equal to 0.8, R^2-adjusted was greater than or equal to 0.7, AAD was around 0, and finally, the A_f and B_f were closed to 1.[27] As shown in Table 4, the coefficients of linear terms (x_1, x_2, and x_3) and quadratic terms (x_1^2, x_2^2, and x_3^2) were shown to be significant for yield, clarity, TSS, titratable acidity and pH. The interaction x_1x_2 term was found to be significant on clarity and titratable acidity, while interaction x_1x_3 was found to be significant for yield, clarity, and titratable acidity, and finally, the pH, TSS, and titratable acidity were affected significantly by the interaction x_2x_3. The strong significance of interactions in this study justifies

Table 3 Doehlert design of coded, real variables and experimental responses

Coded values			Real values			Responses				
Incubation time x_1	Enzyme concentration x_2	Incubation temperature x_3	Incubation time (min) X_1	Enzyme concentration (%) X_2	Incubation temperature (°C) X_3	Yield (%)	pH	Clarity (%T)	Total soluble solids (TSS) (°Brix)	Titratable acidity (% MAE)
0	0	0	105	0.055	45.0	73.67 ± 0	3.76 ± 0	69.8 ± 4.95	7.4 ± 0.14	0.447 ± 0
0	0	0	105	0.055	45.0	73 ± 0.47	3.775 ± 0.01	71.51 ± 4.30	7.5 ± 0.28	0.447 ± 0
0	0	0	105	0.055	45.0	73.33 ± 0	3.77 ± 0.01	67.68 ± 2.47	7.4 ± 0.14	0.447 ± 0
0	0	0	105	0.055	45.0	72.66 ± 0	3.77 ± 0.01	70.68 ± 1.67	7.4 ± 0.14	0.467 ± 0
0	0	0	105	0.055	45.0	74 ± 1.64	3.76 ± 0.01	68.98 ± 3.70	7.5 ± 0.18	0.447 ± 0.03
−1	0	0	30	0.055	45.0	71.5 ± 1.41	3.825 ± 0.01	62.8 ± 2.78	7.1 ± 0	0.424 ± 0.03
1	0	0	180	0.055	45.0	75 ± 0	3.74 ± 0.01	78.82 ± 2.30	7.4 ± 0	0.469 ± 0
−0.5	−0.866	0	67.5	0.01513	45.0	71.67 ± 0	3.77 ± 0	48.69 ± 3.67	7.1 ± 0.14	0.411 ± 0.03
0.5	−0.866	0	142.5	0.01513	45.0	74 ± 0.94	3.74 ± 0.01	77.47 ± 1.06	7.3 ± 0.28	0.467 ± 0.06
−0.5	0.866	0	67.5	0.09487	45.0	74.67 ± 0	3.775 ± 0.01	83.21 ± 3.52	7.4 ± 0	0.447 ± 0
0.5	0.866	0	142.5	0.09487	45.0	75 ± 1.77	3.72 ± 0.04	84.43 ± 0.41	7.5 ± 0.14	0.409 ± 0
−0.5	−0.289	0.816	67.5	0.041995	53.16	72.63 ± 0.47	3.77 ± 0.01	64.19 ± 3.25	7.3 ± 0	0.500 ± 0.03
0	0.577	0.816	105	0.080965	53.16	74.5 ± 0	3.755 ± 0.01	77.15 ± 4.64	7.2 ± 0.28	0.438 ± 0.31
0.5	−0.289	0.816	142.5	0.041995	53.16	72.5 ± 1.89	3.75 ± 0	63.95 ± 5.34	7.4 ± 0	0.469 ± 0.03
−0.5	0.289	−0.816	67.5	0.068005	36.84	71.67 ± 0.94	3.78 ± 0	70.15 ± 1.92	7.1 ± 0	0.424 ± 0.03
0	−0.577	−0.816	105	0.029035	36.84	73 ± 0.94	3.77 ± 0	72.52 ± 2.49	7.1 ± 0.14	0.433 ± 0.06
0.5	0.289	−0.816	142.5	0.068005	36.84	74.33 ± 1.88	3.735 ± 0.01	87.94 ± 6.14	7.3 ± 0.14	0.502 ± 0

Table 4 Coefficients of the second order polynomial models for the responses (yield, pH, clarity, TSS, titratable acidity), *P* values and validation tools (R^2, R^2-adjusted, AAD, A_f, and B_f)

	Yield (%)		pH		Clarity (%T)		TSS (°Brix)		Titratable acidity (% MAE)	
Source	Coefficient	*P* Values	Coefficient	*P* Values	Coefficient	*P* Values	Coefficient	*P* Values	Coefficient	*P* Values
x_0	73.332	NA	3.767	NA	69.73	NA	7.44	NA	0.451	NA
x_1	1.524	0.0001	−0.04	0.0001	9.95	0.0010	0.15	0.0107	0.019	0.0001
x_2	1.126	0.0006	−0.005	0.1420	11.61	0.0004	0.10	0.0432	−0.007	0.0036
x_3	0.129	0.4198	−0.002	0.5629	−5.17	0.0161	0.0817	0.0831	0.0098	0.0148
x_{11}	−0.082	0.7685	0.0155	0.0655	1.08	0.6824	−0.19	0.0451	−0.0045	0.0580
x_{22}	0.667	0.0484	−0.025	0.0060	4.39	0.1376	−0.086	0.2812	−0.0208	0.0023
x_{33}	−0.487	0.1145	−0.008	0.1599	3.02	0.2754	−0.24	0.0161	0.0213	0.0056
x_{12}	−1.129	0.0192	−0.014	0.1168	−15.55	0.0051	−0.0564	0.5376	−0.053	0.0003
x_{13}	−2.109	0.0026	0.010	0.2792	−16.56	0.0068	−0.081	0.4423	−0.0856	0.0001
x_{23}	1.105	0.0331	0.019	0.0695	3.47	0.3950	−0.213	0.0798	−0.0484	0.0003
R^2	0. 938		0.974		0.969		0.901		0.959	
R^2-adjusted	0.941		0,928		0.930		0.775		0.906	
AAD	0.003		0.001		0.023		0.007		0.021	
A_f	1.003		1.001		1.023		1.007		1.022	
B_f	1.000		1.000		1.001		1.000		0.988	

x is the coefficient of the equations for each mathematical model; x_0 is the constant term, x_1, x_2, and x_3 are the linear effects (1, 2, 3 for incubation time, enzyme concentration and incubation temperature respectively), x_{11}, x_{22}, and x_{33} are quadratic effects and x_{12}, x_{13}, and x_{23} are the interactions. TSS, total soluble solids; AAD, absolute average deviation; A_f, exactitude factor; B_f, bias factor; MAE, malic acid equivalent; NA, not available.

the use of RSM as an optimization tool since the one-variable-at-a-time method does not allow the investigation of the contribution of interactive effects, which makes it difficult to determine optimum values. It is worth noting that as for all the responses, incubation time (x_1) had the most significant effect, followed by enzyme concentration (x_2) and incubation temperature (x_3), respectively.

The high R^2 (coefficient of determination) for yield, pH, clarity, TSS and titratable acidity of 0.938, 0.974, 0.969, 0.901 and 0.959 indicated that the variables successfully explained 93.8%, 97.4%, 96.9%, 90.1% and 95.9% of the variation in the models respectively, denoting the models are practical. Nonetheless, the value of each R^2 was close to that of its respective R^2-adjusted value, hence corroborating the high explanatory power of the regression models used in this study. The results represented in Table 4 also revealed that the five models were significant and suitable for the adequate prediction of yield, pH, clarity, TSS, and titratable acidity responses within the variable ranges chosen in this study. Deleting all non-significant terms, the regression models for yield, pH, clarity, TSS, and titratable acidity are listed as follows:

$$Y_1 = 73.332 + 1.524x_1 + 1.126x_2 + 0.667x_2^2 - 1.129x_1x_2 - 2.109x_1x_3 + 1.105x_2x_3 \quad (3)$$

$$Y_2 = 3.767 - 0.04x_1 - 0.025x_2^2 \quad (4)$$

$$Y_3 = 69.73 + 9.95x_1 + 11.61x_2 - 5.17x_3 - 15.55x_1x_2 - 16.56x_1x_3 \quad (5)$$

$$Y_4 = 7.44 + 0.15x_1 + 0.1x_2 - 0.19x_1^2 - 0.2414x_3^2 \quad (6)$$

$$Y_5 = 0.451 + 0.019x_1 - 0.007x_2 + 0.009x_3 - 0.020x_2^2 + 0.002x_3^2 - 0.053x_1x_2 - 0.085x_1x_3 - 0.048x_2x_3 \quad (7)$$

where, Y_1 = yield (%), Y_2 = pH, Y_3 = clarity (%T), Y_4 = TSS (°Brix), Y_5 = titratable acidity (% MAE), x_1 = incubation time, x_2 = enzyme concentration and x_3 = incubation temperature.

3.4 Analysis of RSM

Table 4 shows that incubation time had a significant impact on all the responses. An increase in incubation time resulted in a significant increase in the yield, clarity, and titratable acidity and a significant decrease in the pH ($P < 0.05$). Results shown in Fig. 1(a) were obtained by fixing enzyme concentration (x_2) at 0.015% and the incubation temperature (x_3) at 36.84 °C, while varying the incubation time (x_1); Fig. 1(b) by fixing incubation time (x_1) at 30 min and incubation temperature (x_3) at 36.84 °C while varying enzyme concentration (x_2) and Fig. 1(c) was obtained by fixing the incubation time (x_1) at 30 min and enzyme concentration (x_2) at 0.015% with a variation in incubation temperature (x_3).

3.4.1 *Effect of variables on yield*

An increase of incubation time from 30 to 180 min, led to a significant increase from 68.90 to 77.35% for yield (Fig. 1(a)). Juice yield while increasing the incubation time was paired with a more pronounced action of the pectinase. Indeed, pectinase acts by cleavage of the pectin constituting the cell wall of the soursop pulp at the α-1,4-glucosidic bonds into monomers of galacturonic acid, resulting in the breakdown of cell walls and a release of intracellular liquids containing solutes that migrate from intracellular environment to extracellular environment and hence increased the extraction yield of juice.[18] It has been reported that prolonged incubation resulted in a higher extraction yield of fruit juice.[15,18] Increase of enzyme concentration from 0.015% to 0.095% induced a significant increase (Fig. 1(b)) in yield from 68.90% to 71.25%. Since the enzyme concentration is very low compared to that of the substrate, the reaction rate is directly proportional to the concentration of the enzyme. That is, the reaction rate increased as enzyme concentration increased. In other words, the significant increase in yield could be related to the rate of hydrolysis of the pectin following the increase in pectinase concentration. Similar observations have been reported[15,34] for pectin-based pulps treated with pectinase. The impact of

interaction x_1x_3 (incubation time/incubation temperature) as shown in Fig. 1(f) revealed that the higher the incubation time and incubation temperature within the experimental range, the better and faster the pulp hydrolysis resulting in an increase in the juice extraction yield because of pulp degradation by pectinase followed by juice release.[15]

3.4.2 *Effect of variables on pH*

There was a significant decrease in pH value from 3.81 to 3.74 when the incubation time was increased from 30 to 180 min, which could be attributed to the liberation of the carboxyl groups and other organic acids following the enzymatic hydrolysis of pectin.[35] This decrease in pH with an increase in extraction time is similar to the findings of Makebe *et al.*[27] and Yusof and Ibrahim.[15] It is observed from Fig. 1(i) that the impact of the interaction x_2x_3 (enzyme concentration/incubation temperature) was significant on pH. A simultaneous increase in the values of both factors contributed to a decrease in the pH value, and this is obviously due to the release of galacturonic and other organic acids.[35]

3.4.3 *Effect of variables on clarity*

Increasing the incubation time (30 to 180 min) led to a significant increase in clarity from 35.76 to 100% (Fig. 1(a)). During enzymatic treatment, pectinases hydrolyze pectin molecules over time, facilitating the formation of protein–pectin complexes, and the elimination of these colloidal particles in juice contributes to an increase of juice clarity.[15,34] This result is similar to that reported[16,26] whereby the effect of pectinase on the extraction yield of banana juice was studied. Increasing the concentration of pectinase (Fig. 1(b)) could increase the clarity of juice, which in this study, increased from 35.76%T to 77.90%T when enzyme concentration was increased from 0.015% to 0.095%, thus suggesting the formation of larger aggregates as earlier explained and thereby settling,[34] eventually increasing the clarity of the juice. The increase in juice clarity from 35.77%T to 49.52%T with incubation temperature (36.84 to 53.16 °C) (Fig. 1(c)) is explained by the fact that the optimal temperature of pectinases is between 40 and 55 °C.[36] An increase in temperature in this range allows a more efficient pectinase action upon the hydrolysis of pectin, and thus an increasingly clear juice. The significant impact of interaction x_1x_2 (incubation time/enzyme concentration) is presented in Fig. 1(d) with a significant increase in clarity (from 35.77%T to 97.89%T), indicating a synergistic effect between both factors. This is quite normal because incubation time and pectinase concentration are considered as key parameters for pectin hydrolysis. Knowing the product of the reaction of pectinase on pectin and the consequences on the juice clarity, it is therefore evident to obtain an increase of both responses. This was obtained at the same incubation temperature of 36.84 °C. As shown in Fig. 1(g), interaction X_1X_3 (incubation time/incubation temperature) had a significant increase in clarity, and this could be due to the better decantation of trub.[35]

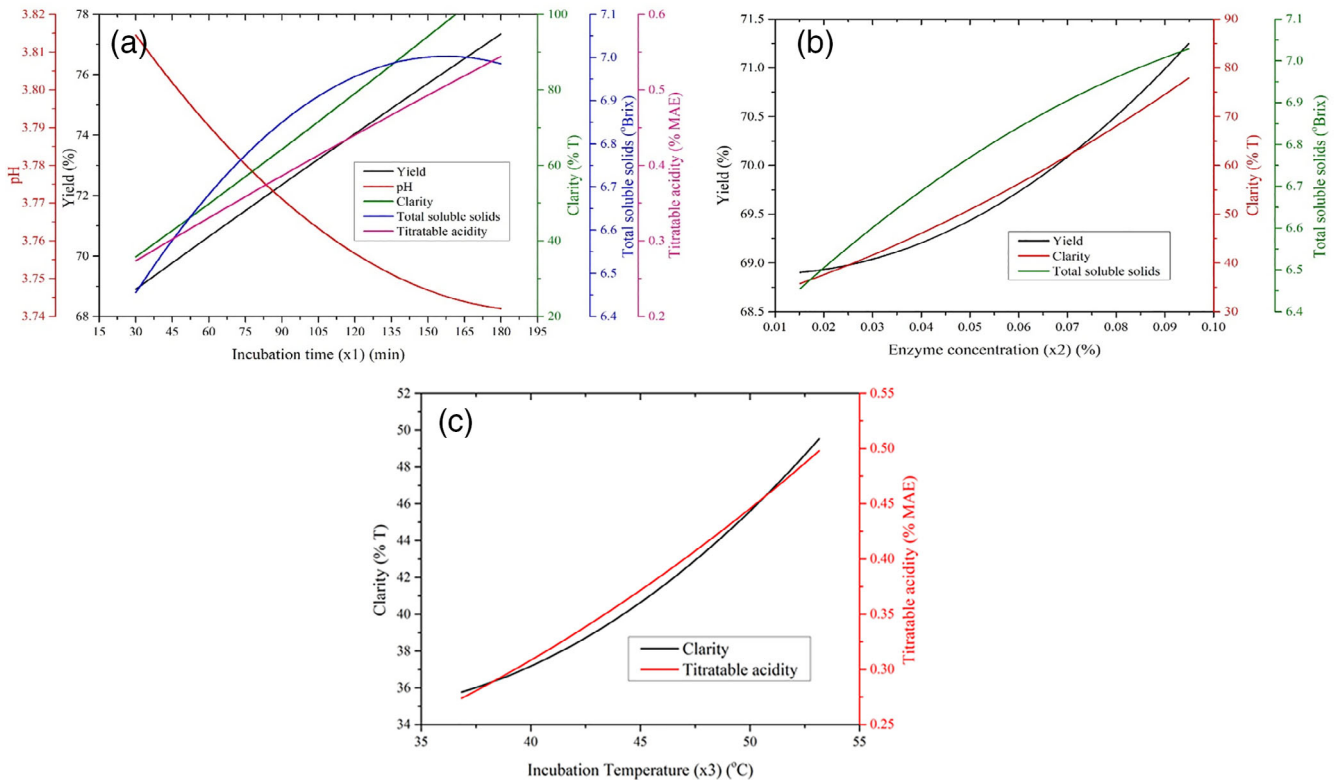

Figure 1 Evolution of responses as a function of factors: (a) incubation time (enzyme concentration and incubation temperature fixed respectively at 0.015% and 36.84 °C); (b) enzyme concentration (incubation time and incubation temperature fixed respectively at 30 min and 36.84 °C); (c) incubation temperature (incubation time and enzyme concentration fixed respectively at 30 min and 0.015%); mesh plot of clarity (d) and titratable acidity (e) as a function of incubation time (x_1) and enzyme concentration (x_2) (incubation temperature fixed at 36.84 °C); yield (f), clarity (g) and titratable acidity (h) as a function of incubation time (x_1) and incubation temperature (x_3) (enzyme concentration fixed at 0.015%); pH (i), total soluble solids (j) and titratable acidity (k) as a function of enzyme concentration (x_2) and incubation temperature (x_3) (incubation time fixed at 30 min).

(Figure continues on next page.)

(Figure continued from previous page.)

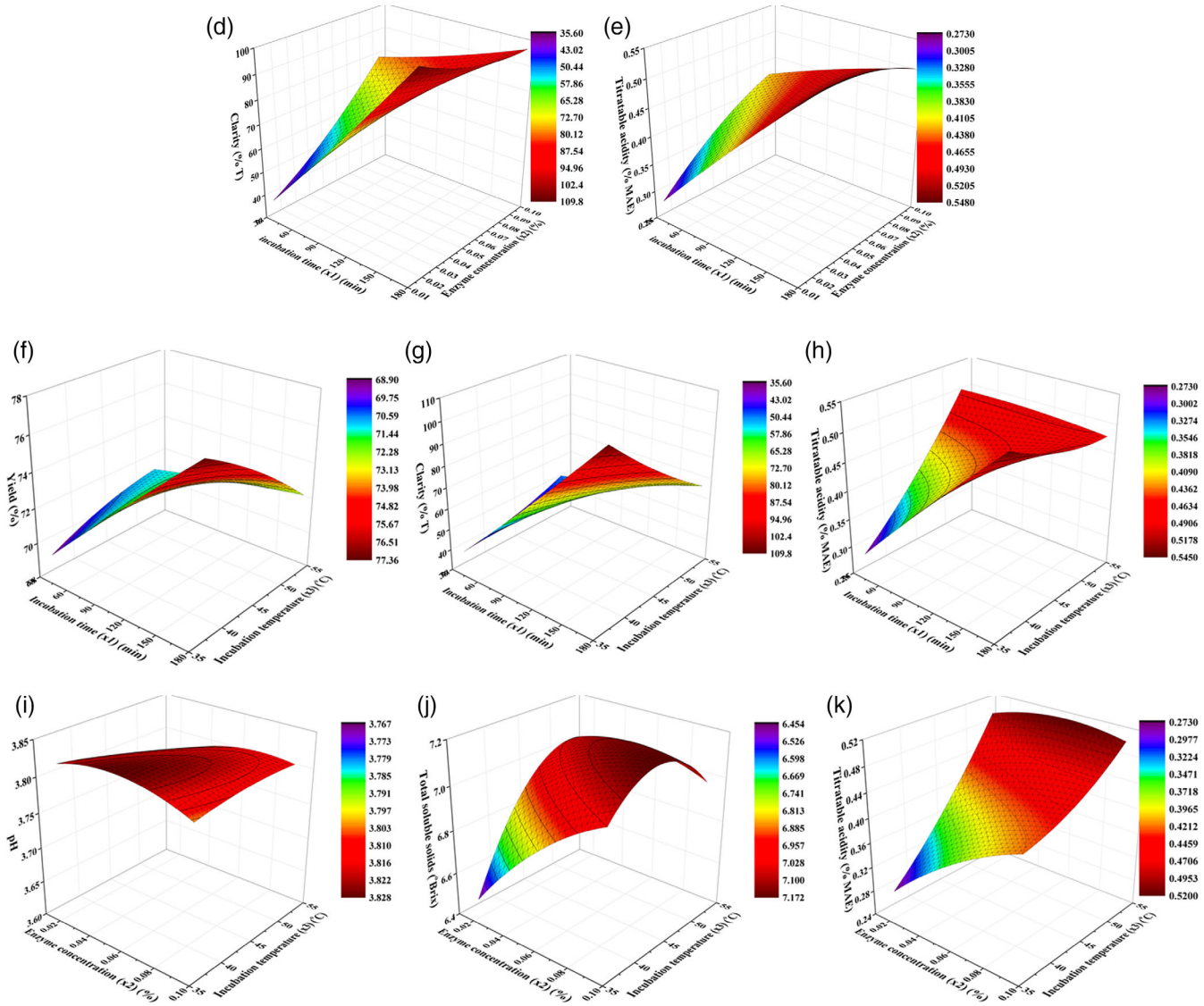

Figure 1 (Continued)

3.4.4 *Effect of variables on TSS*

As for TSS, an increase in incubation time from 30 to 52.66 min (Fig. 1(a)), contributed to an increase in the TSS value from 6.45 °Brix to 7.00 °Brix, meanwhile further increase in incubation time from 52.66 to 180 min yielded constant TSS (Fig. 1(a)). This could be explained by the extensive hydrolysis of cellular pectin, resulting in increased release of compounds such as sugars[35] and other components that are soluble solids. A plateau was observed for the TSS towards the end of the prolonged incubation time. An increase in TSS from 6.45 °Brix to 7.03 °Brix with an increase in enzyme concentration (Fig. 1(b)) could be attributed to the release of significant soluble solids components into the medium following hydrolysis.[27] The impact of the interaction x_2x_3 (enzyme concentration/incubation temperature) represented in Fig. 1(j) was significant on TSS, which was due to the accumulation of the soluble solids formed upon hydrolysis.[35]

3.4.5 *Effect of variables on titratable acidity*

A significant increase in titratable acidity from 0.27 to 0.55% MAE was observed with an increase of incubation time from 30 to 180 min (Fig. 1(a)). Commercial pectinase is a mixture of several enzymes, including polygalacturonase and pectin methylesterase. Hydrolysis of pectin during long incubation time led to the release of galacturonic acids and other organic acids due to the action of these two enzymes, thus increasing the titratable acidity of the juice. Several authors have observed this phenomenon.[15,27,37] Organic acids play a relevant role in the processing of fruit juice. It serves as a mild preservative, contributes to the flavor development via a balanced sugar/acid ratio, and stimulates saliva secretion through a thirst-quenching effect.[9,14,38] The increase in titratable acidity (from 0.274% MAE to 0.498% MAE) is justified by the fact that further hydrolysis of pectin by pectinase with increasing incubation temperature (Fig. 1(c)) permitted

a greater release of galacturonic acids and other organic acids in the juice. The interaction x_1x_2 (incubation time/enzyme concentration) as presented in Fig. 1(e) contributed to a significant increase in the titratable acidity from 0.273% MAE to 0.508% MAE meanwhile, for the interaction x_1x_3 (incubation time/incubation temperature) shown in Fig. 1(h) and the interaction x_2x_3 (enzyme concentration/incubation temperature) represented in Fig. 1(k), it was observed that the factors concerned with the interactions increased the titratable acidity simultaneously because of the release of galacturonic acid and other organic acids.[35]

3.5 Optimization and verification of the predicted optimum

The optimum conditions for juice yield, pH, clarity, TSS, and titratable acidity were found to be different. Therefore, there was a need to find a predicted composite optimum from the models, which should take into account all responses (yield, pH, clarity, TSS, and titratable acidity). The model simulation generated theoretical optimum conditions for the extraction of soursop juice as follows: 172.22 min, 0.0398%, and 42.15°C for incubation time, enzyme concentration, and incubation temperature, respectively. Under theoretical conditions, the predictive responses were 75.23%, 3.746, 87.06%T, 7.35 °Brix, and 0.44% MAE for juice yield, pH, clarity, TSS, and titratable acidity, respectively. Using the optimum conditions, the following experimental results were obtained; 77.33%, 3.64, 94.95%T, 7.5 °Brix, and 0.46% MAE for juice yield, pH, clarity, TSS and titratable acidity, respectively. These results were close to that predicted by the model simulation, which implies that each model was quite precise in its prediction and also confirmed the validation done using statistical tools.

3.6 Morphological analysis

In Fig. 2(a), soursop presented a non-uniform spherical surface, and this was ascribed to the breeding of ice in intermolecular spaces of the soursop during lyophilization at the point of freezing of the free water, limiting the molecular movement of polysaccharides linked to bound water. A similar observation was also reported for guava structure.[39] In contrast, hydrolyzed soursop (Fig. 2(b)) presented a ruptured, coarse, and wrinkled surface compared to unhydrolyzed soursop (Fig. 2(a)), which had all its fibrous structures maintained. The damaged structure of hydrolyzed soursop is linked to pectinase hydrolysis of pectic substances like polygalacturans, which make up the middle lamella and are bound by α-1-4-glucosidic bonds of galacturonic acid units.[40] The hydrolysis of pectic substances caused the collapse of the middle lamella and the loss of structural matrix of the cell walls resulting in the release of more solutes from soursop and increase of yield of soursop juice extraction. Therefore, the destruction of soursop cell walls with the help of pectinase was

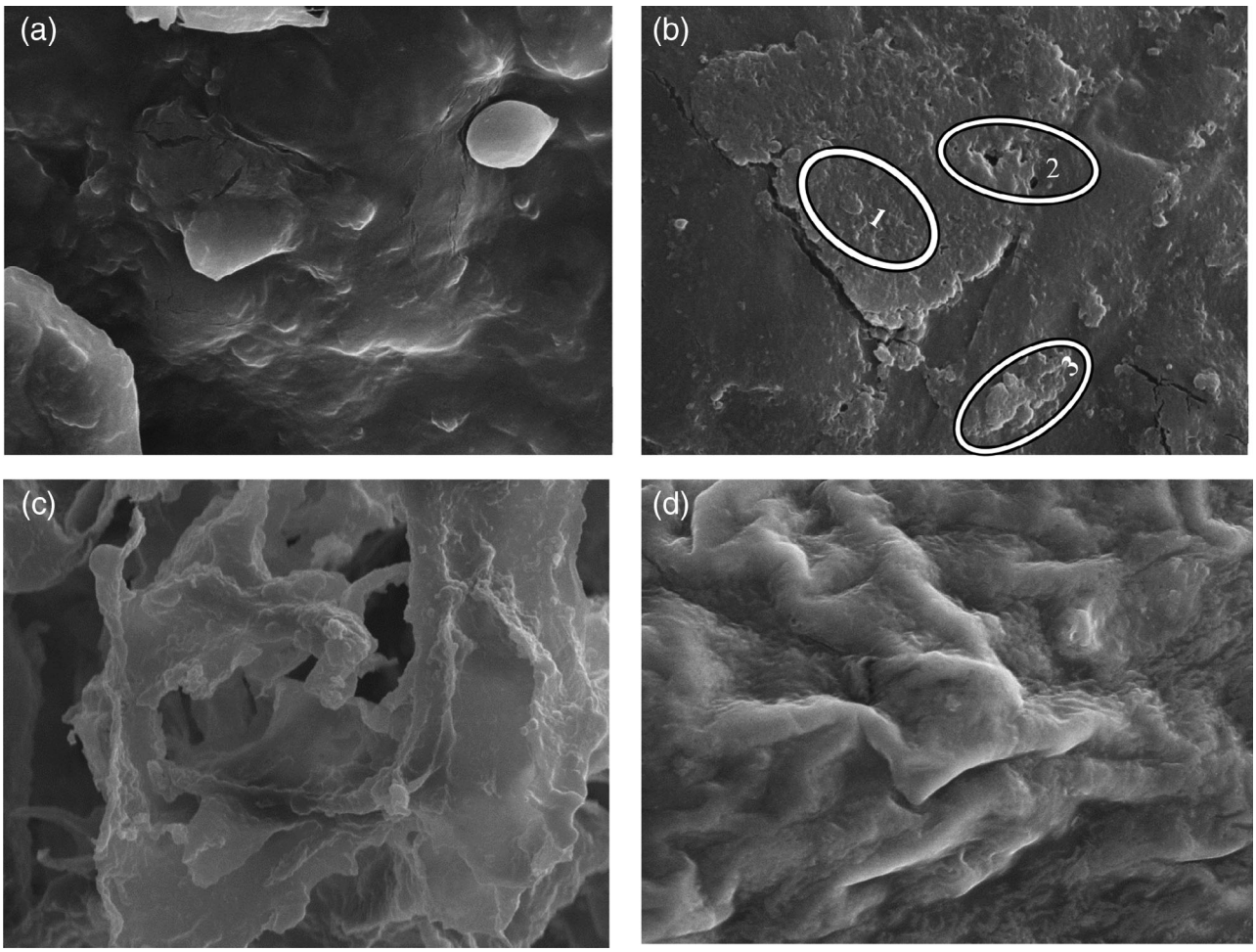

Figure 2 Micrographs of untreated soursop (a), hydrolyzed soursop (b), soursop pectin (c) and hydrolysed soursop pectin (d). 1, coarse; 2, ruptured; 3, wrinkled.

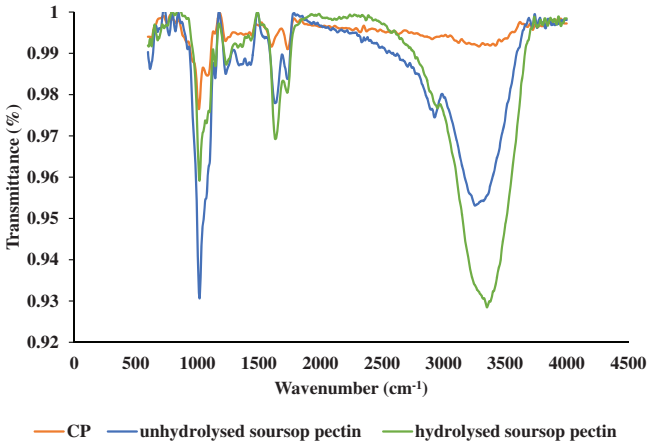

Figure 3 FTIR spectra for unhydrolyzed soursop pectin, hydrolyzed soursop pectin and commercial citrus pectin.

beneficial for releasing solutes, which were previously detained in the plant cell structure.

The image of pectin extracted from untreated soursop (Fig. 2(c)) was found to be porous. This is presumed to be as a consequence of the high incubation temperature (90°C), which generates a disintegration in the structure, leading to a thinner surface.[41] Pectin from hydrolyzed soursop is of the low methoxyl type, and its destruction is responsible for the whirling rough surface presented in Fig. 2(d). The morphology, as mentioned earlier, is predicted to be a result of the removal of methyl esters leading to degradation of the galacturonic acid linkages and calcium-mediated crosslinks between pectins, which corroborates to total structural rupture.[42]

3.7 Structural analysis

The FTIR spectra of commercial pectin, pectin from untreated soursop pulp and hydrolyzed soursop pulp are shown in Fig. 3. FTIR spectra of different pectin samples have characteristic peaks at 3390.6, 2939.0, 1749.0, and 1052.1 cm^{-1} corresponding to –OH, –CH, C═O of ester, and acid, and –COC– stretching of galacturonic acid (Supplementary Table 1).[43,44] The intensity of bands between 3400–2900 cm^{-1} was higher in pectin from hydrolyzed soursop, confirming the hydrolysis of glycosidic bonds and exposition of free –OH to a greater extent. This is in agreement with the findings of Xu *et al.*,[45] who obtained broad absorption peaks at 3410 cm^{-1} (hydroxyl groups) and weak bands at 2920 cm^{-1} (C—H stretching) from jackfruit pectin. Likewise, Manrique and Lajolo[46] also reported the same stretching at 3400 cm^{-1} and 2930 cm^{-1} bands for pectin isolated from ripening papaya fruit. The results obtained in this work for commercial pectin and untreated soursop pectin are in accordance with the findings of Xu *et al.*[45] whose FTIR data confirmed that methyl-esterified forms existed predominantly in pectin samples. Other studies revealed the assignment of C═O stretching vibration of the methyl-esterified carboxyl groups of bands at 1750 cm^{-1} for soy hull pectin,[43] at 1746 cm^{-1} for jackfruit pectin[45] and 1737 cm^{-1} for the cell wall pectin fraction of ripe strawberry fruit .[47] FTIR spectra of both samples showed a good match with the spectrum of commercial pectin. The relatively weak intensity of the Raman bands at 1470, 1183, and 1165 cm^{-1} evidenced that pectins from hydrolyzed soursop and untreated soursop were acetylated (Fig. 3). The region of 1200 to 1000 cm^{-1} contained skeletal C—O and C—C vibration bands of glycosidic bonds and pyranoid ring, and this is in agreement with the works of Kalapathy and Proctor.[43] The band intensity of ring vibrations and C—O stretching was accentuated in pectin from hydrolyzed soursop. Moreover, the intensity of band of glycosidic bonds was higher in commercial and untreated soursop pectin, evidencing the breakdown of glycosidic bonds by pectinase during liquefaction. Therefore, FTIR spectra revealed evidence of the breakdown of soursop pulp pectin during liquefaction.

4 CONCLUSION

The current study optimized the pectinase-assisted extraction of soursop juice. The optimum conditions of the soursop juice extraction process obtained were 172 min of incubation time, 0.04% (*w/w*) of enzyme concentration, and incubation temperature at 42.9 °C. The combination of these optimal conditions resulted in 75.2% yield, pH of 3.75, TSS of 7.35 °Brix, 87.06%T clarity, and titratable acidity of 0.46% MAE. With the optimal conditions, the numerical predictions were similar to the experimental data obtained, which ranged from 0.905 to 0.987, thus confirming the validity of the models. The morphological analysis using scanning electron microscopy (SEM) revealed that pectinase hydrolyzed the pectin in soursop and improved the juice extraction process. The FTIR spectra of pectin from untreated and hydrolyzed soursop indicated bands between 1790 and 1610 cm^{-1}, representing spectral identification of galacturonic acid with a higher intensity seen for the hydrolyzed soursop pectin. This structural variation was attributed to the hydrolysis of the soursop pectin. The study provided the optimized conditions for pectinase-assisted extraction of soursop juice, which could be one of the promising methods for the value addition of soursop.

ACKNOWLEDGEMENTS

The authors acknowledge and thank The World Academy of Science (TWAS) Italy and the Council of Scientific and Industrial Research (CSIR) India for granting Makebe Calister Wingang with the Sandwich Postgraduate Fellowship Award and the directors of CSIR-National Institute for Interdisciplinary Science and Technology (CSIR-NIIST), Trivandrum, Kerala, India and National Advanced School of Agro-Industrial Sciences (ENSAI), University of Ngaoundere, Cameroon, for providing the necessary facilities for the accomplishment of this work.

CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

SUPPORTING INFORMATION

Supporting information may be found in the online version of this article.

REFERENCES

1 Kossouoh C, Moudachirou M, Adjakidje V, Chalchat J-C, Figuérédo G. Essential oil chemical composition of *Annona muricata* L. leaves from Benin. *J Essent Oil Res* **19**(4):307–9 (2007).
2 Patel S, Patel JK. A review on a miracle fruits of *Annona muricata*. *J Pharmacogn Phytochem* **5**(1):137–48 (2016).
3 Sanusi SB, Abu Bakar MF. Soursop *Annona muricata*. In: *Exotic Fruits*. Amsterdam: Elsevier; p. 391–5 (2018).

4 Badrie N, Schauss AG. Soursop (*Annona muricata* L.). In: *Bioactive Foods in Promoting Health*. Amsterdam: Elsevier; 2010; p. 621–43.
5 Adefegha SA, Oyeleye SI, Oboh G. Distribution of phenolic contents, antidiabetic potentials, antihypertensive properties, and antioxidative effects of soursop (*Annona muricata* L.) fruit parts *in vitro*. *Biochem Res Int* **2015**:1–8 (2015).
6 Moreau D, Huchot E, Gazaille V, Rossanaly-Vasram R, Andre M. Automédication par *Annona muricata* L. (corossol) à visée anticancéreuse à la Réunion. *Rev Mal Respir* **35**(9):948–55 (2018).
7 Costa J de P da, Rocha ÉM de FF, Costa JMC da. Study of the physicochemical characteristics of soursop powder obtained by spray-drying. *Food Sci Technol Camp* **34**(4):663–6 (2014).
8 Umme A. Microbial and enzymatic changes in natural soursop puree during storage. *Food Chem* **65**(3):315–22 (1999).
9 Chang LS, Karim R, Sabo Mohammed A, Mohd Ghazali H. Characterization of enzyme-liquefied soursop (*Annona muricata* L.) puree. *LWT – Food Sci Technol* **94**:40–9 (2018).
10 Anaya-Esparza LM, Velázquez-Estrada RM, Sayago-Ayerdi SG, Sánchez-Burgos JA, Ramírez-Mares MV, García-Magaña ML, et al. Effect of thermosonication on polyphenol oxidase inactivation and quality parameters of soursop nectar. *LWT – Food Sci Technol* **75**:545–51 (2017).
11 Quintana SE, Pardo A, Castellon S, Gonzalez-Cuello RE, Garcia-Zapateiro LA. Viscous characterization of soursop (*Annona muricata*) jam with blend of hydrocolloids. *Contemp Eng Sci* **11**(26):1269–77 (2018).
12 Abbo ES, Olurin TO, Odeyemi G. Studies on the storage stability of soursop (*Annona muricata* L.) juice. *Afr J Biotechnol* **5**(19):1808–12 (2006).
13 Dias D da RC, Barros ZMP, Carvalho CBO de, Honorato FA, Guerra NB, Azoubel PM. Effect of sonication on soursop juice quality. *LWT – Food Sci Technol* **62**(1):883–9 (2015).
14 Ndife J, Kwaya PJ, Bello S. Production and evaluation of storage changes in soursop-juice. *Asian J Agric Food Sci* 2014; **02**(05): 425–33.
15 Yusof S, Ibrahim N. Quality of soursop juice after pectinase enzyme treatment. *Food Chem* **51**:8–88 (1994).
16 Sagu ST, Nso EJ, Karmakar S, De S. Optimisation of low temperature extraction of banana juice using commercial pectinase. *Food Chem* **151**:182–90 (2014).
17 Kumar S. Role of enzymes in fruit juice processing and its quality enhancement. *Adv Appl Sci Res* **6**(6):114–24 (2015).
18 Lee WC, Yusof S, Hamid NSA, Baharin BS. Optimizing conditions for hot water extraction of banana juice using response surface methodology (RSM). *J Food Eng* **75**(4):473–9 (2006).
19 Ninga KA, Sengupta S, Jain A, Desobgo ZSC, Nso EJ, De S. Kinetics of enzymatic hydrolysis of pectinaceous matter in guava juice. *J Food Eng* **221**:158–66 (2018).
20 Anuradha K, Naga Padma, Venkateshwar S, Gopal R. Mango juice clarification with polygalacturonase produced by *Aspergillus awamori* MTCC 9166 – optimization of conditions. *Int Food Res J* **23**(1):147–51 (2016).
21 Chang LS, Karim R, Abdulkarim SM, Ghazali HM. Production and characterization of enzyme-treated spray-dried soursop (*Annona muricata* L.) powder. *J Food Process Eng* **41**(5):1–12 (2018).
22 Montgomery DC. Response Surface Methods and Other Approaches to Process Optimization. *Design and Analysis of Experiments*. 5th ed.. New York: John Wiley & Sons; pp. 684 (2001).
23 Goupy J, Creighton L. Plans pour surfaces de réponse . *Introduction aux plans d'expériences*. 3rd ed., Paris: Dunod; pp. 324 (2006).
24 Sharma HP, Patel H, Sugandha. Enzymatic added extraction and clarification of fruit juices – a review. *Crit Rev Food Sci Nutr*. **57**(6):1215–27 (2017).
25 Singh A, Kumar S, Sharma HK. Effect of enzymatic hydrolysis on the juice yield from Bael fruit (*Aegle marmelos* Correa) pulp. *Am J Food Technol* **7**(2):62–72 (2012).
26 Rai P, Majumdar GC, DasGupta S, De S. Optimizing pectinase usage in pretreatment of mosambi juice for clarification by response surface methodology. *J Food Eng* **64**(3):397–403 (2004).
27 Makebe C, Desobgo Z, Nso E. Optimization of the juice extraction process and investigation on must fermentation of overripe Giant horn plantains. *Beverages* **3**(4):19 (2017).
28 AOAC, Association of Official Analytical Chemists. 18th ed., 2005.08. (2005).
29 Ranganna S. Pectin. *Handbook of Analysis and Quality Control for Fruits and Vegetable Products*. 2nd ed., New Delhi: Tata Mcgrawhill; pp. 1103 (2007).
30 Pinto AC de Q, Cordeiro MCR, De Andrade SRM, Ferreira FR, De C. Filgueiras HA, Alves RE, Kinpara DI. *Fruits for the Future 5: Annona Species*. Southampton: Centre for Underutilised Crops; pp. 268 (2005).
31 Orsi DC, Carvalho VS, Nishi ACF, Damiani C, Asquieri ER. Use of sugar apple, atemoya and soursop for technological development of jams–chemical and sensorial composition. *Ciênc Agrotec*, **36**(5): 560–6 (2012).
32 Liew SQ, Chin NL, Yusof YA. Extraction and characterization of pectin from passion fruit peels. *Agric Agric Sci Procedia* **2**:231–6 (2014).
33 Kamble PB, Gawande S, Patil TS. Extraction of pectin from unripe banana peel. *Int Res J Eng Technol* **4**(7):2259–64 (2017).
34 Sin HN, Yusof S, Sheikh Abdul Hamid N, Rahman RAbd. Optimization of enzymatic clarification of sapodilla juice using response surface methodology. *J Food Eng* **73**(4):313–9 (2006).
35 Bitange NT, Zhang W, Shi-Ying X, Wenbin Z. The influence of a pectinase and pectinase/hemicellulases enzyme preparations on percentage pineapple juice recovery, particulates and sensory attributes. *Pak J Nutr* **8**(8):1184–9 (2009).
36 Karangwa E, Khizar H, Rao L, Nshimiyimana DS, Foh MBK, Li L, et al. Optimization of processing parameters for clarification of blended carrot-orange juice and improvement of its carotene content. *Adv J Food Sci Technol* **2**(5):268–278 (2010).
37 Nguyen C, Nguyen H. The quality of mulberry juice as affected by enzyme treatments. *Beverages* **4**(2):41 (2018).
38 Umme A, Asbi BA, Salmah Y, Junainah AH, Jamilah B. Characteristics of soursop natural puree and determination of optimum conditions for pasteurization. *Food Chem* **58**(1–2):119–24 (1997).
39 Osorio C, Carriazo JG, Barbosa H. Thermal and structural study of guava (*Psidium guajava* L) powders obtained by two dehydration methods. *Quím Nova* **34**(4):636–40 (2011).
40 Platt-Aloia KA, Thomson WW, Young RE. Ultrastructural changes in the walls of ripening avocados: transmission, scanning, and freeze fracture microscopy. *Bot Gaz* **141**(4):366–73 (1980).
41 Zhongdong L, Guohua W, Yunchang G, Kennedy JF. Image study of pectin extraction from orange skin assisted by microwave. *Carbohydr Polym* **64**(4):548–52 (2006).
42 Devaux MF, Barakat A, Robert P, Bouchet B, Guillon F, Navez B, Lahaye M Mechanical breakdown and cell wall structure of mealy tomato pericarp tissue. *Postharvest Biol Technol.* **37**(3):209–21 (2005).
43 Kalapathy U, Proctor A. Effect of acid extraction and alcohol precipitation conditions on the yield and purity of soy hull pectin. *Food Chem* **73**(4):393–6 (2001).
44 Karaca B, Bozacı E, Demir A, Ozdogan E, Seventekin N. Effects of enzymatic treatments on surface morphology and chemical structure of linen fabrics. *J Appl Polym Sci* **125**(1):793–7 (2012).
45 Xu S-Y, Liu J-P, Huang X, Du L-P, Shi F-L, Dong R, et al. Ultrasonic-microwave assisted extraction, characterization and biological activity of pectin from jackfruit peel. *LWT – Food Sci Technol* **90**:577–82 (2018).
46 Manrique GD, Lajolo FM. FT-IR spectroscopy as a tool for measuring degree of methyl esterification in pectins isolated from ripening papaya fruit. *Postharvest Biol Technol* **25**(1):99–107 (2002).
47 Posé S, Kirby AR, Mercado JA, Morris VJ, Quesada MA. Structural characterization of cell wall pectin fractions in ripe strawberry fruits using AFM. *Carbohydr Polym* **88**(3):882–90 (2012).
48 Szymanska-Chargot M, Zdunek A. Use of FT-IR spectra and PCA to the bulk characterization of cell wall residues of fruits and vegetables along a fraction. *Process Food Biophys* **8**(1):29–42 (2013).

