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Comparative extraction of bromelain and bioactive peptides from pineapple byproducts by ultrasonic- and microwave-assisted extractions

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Abstract

Ultrasonic-assisted extraction (UAE) and microwave-assisted extraction (MAE) were used for the extraction of bioactive peptides from pineapple byproducts. Independent extraction parameters for UAE were ultrasonic amplitude (60–100%), extraction time (10–30 min), and solvent to material ratio (20–40 mL/g), whereas for MAE independent variables were microwave power (100–300 W), irradiation time (5–15 min), and feed to solvent ratio (1:8–1:12 g/mL). The optimized extraction conditions were 99.96%, 26.83 min, and 20.96 mL/g for UAE and 100 W, 8.99 min, and 1:8 g/mL for MAE. The optimized extraction resulted in total sugar, protein content, and proteolytic activity of 15.71 ± 0.03 mg/mL, 4.01 ± 0.04 mg/mL, and 196.46 ± 3.29 U/mL, respectively, whereas 33.87 ± 0.03 mg/mL, 2.50 ± 0.01 mg/mL, and 154.08 ± 1.49 U/mL for MAE, respectively. Scanning electron microscopy was used to observe the morphology of sample during extraction. Molecular weight determination showed that the major protein band in the extracts (UAE and MAE) was at ~23 kDa which corresponded to bromelain. Furthermore, pineapple crown protein extract obtained by UAE showed high proteolytic activity (>80% relative). The present study indicated that bromelain and other bioactive peptides from pineapple byproducts have potential in food, feed, and pharmaceutical products development.

Practical Applications

The conventional extraction techniques for bioactive compounds are time-consuming and less efficient. The use of advance extraction techniques like microwave- and ultrasonication-assisted extraction techniques can increase the extraction yield with better recovery of bioactive compounds. Pineapple byproducts are rich source of bioactive compounds and bromelain. The effective utilization of pineapple byproducts for the extraction of bromelain and other bioactive peptides will offer an effective solution for minimizing the organic waste and provide an efficient source of bioactive peptides for application in various food products.

1 | INTRODUCTION

Recently, there has been an increased industrial interest in the valorization of various agro-industrial wastes, such as peels, seeds, and other byproducts for food processing industry efficiently for production of value-added ingredients. Recently, the demand for functional food ingredients and nutraceuticals is at rise due to their potential to boost consumer's immune system (Galanakis, 2015; Galanakis et al., 2021). For the development of sustainable food supply chain system, it is essential to effectively extract and utilize bioactive compounds from food processing byproducts (Galanakis, 2020). Plant derived bioactive peptides have been reported to exhibit anti-

inflammatory, antimicrobial, antioxidant, and immunomodulatory activities (Galanakis et al., 2020).

Pineapple (*Ananas comosus*) is one of the most abundantly grown in several tropical and sub-tropical countries. The top 10 pineapple producing countries are Brazil, Thailand, Philippines, Costa Rica, India, Nigeria, Indonesia, China, Mexico, and Colombia (FAOSTAT, 2020). Thailand is currently the fourth largest producer and trading market of pineapple in the world, whereas 80% of pineapple is converted into canned pineapple and refined pineapple juice, whereas the rest 20% is sold domestically (Saenmuangchin & Siripinyanond, 2019). Pineapple is one of the main ingredients in various products such as juice concentrates, jams, squash, jellies, essence, and pickles.

Pineapple fruit comprises only 30% (w/w) of the pulp while the rest (70%) is waste as residue, crown, peel core, and trimmings (Ketnawa, Chaiwut, & Rawdkuen, 2012; Nakthong, Wongsagonsup, & Amornsakchai, 2017). Most of these wastes generated by pineapple processing industry are normally disposed as such. It is now desirable to extract and to utilize these pineapple wastes as source of proteins, peptides and other bioactive compounds, including some valuable proteolytic enzymes including bromelain. The pineapple has been considered as the only source for cysteine protease, bromelain (Grzonka, Kasprzykowski, & Wiczk, 2007; Ketnawa, Rawdkuen, & Chaiwut, 2010). Bromelain also exhibits extensive applications in food and pharmaceutical industries and detergent manufacturing units (Feijoo-Siota & Villa, 2011). Bromelain has been used for meat tenderizing, brewing, baking, and production of protein hydrolysates in food industry applications (Ketnawa et al., 2012).

Conventional extraction methods involve high temperature and prolonged period of extraction time, which increase the risk of degradation of thermo-labile bioactive compounds and results in lesser yield (Pimentel-Moral et al., 2018). The aqueous extraction of value-added compounds from agriculture products is recognized as environment friendly, economical, and without the use of chemical solvents (Sepúlveda, Romaní, Aguilar, & Teixeira, 2018). Nonconventional emerging extraction technologies, such as pulse electric field and ultrasonic-assisted extraction (UAE) can reduce the extraction time, minimize the solvent consumption, improve the yield of bioactive compounds from agriculture products, and maintain the bioavailability and functionality (Barba, Galanakis, Esteve, Frigola, & Vorobiev, 2015). Emerging technologies like UAE are not based on high temperature and use the internal energy transmission to generate the heat which maintains the sensory and functional food attributes (Galanakis, 2021; Zinoviadou et al., 2015). UAE and microwave-assisted extraction (MAE) provide immense advantage especially in terms of lesser extraction time with higher yield. Sonication is a method where sound waves are applied to disrupt plant cell walls which subsequently increase the solvent penetration, resulting in a high extraction yield (Jain & Anal, 2016; Koirala, Prathumpai, & Anal, 2021). MAE is also an innovative method that combines microwave irradiation with organic solvent extraction. MAE is based on the use of microwave energy causing molecular motion by ionic conduction and dipole rotation and has attracted considerable attention due to its shorter extraction time, lower solvent consumption, and higher extraction yield (da Rosa, Vanga, Gariepy, & Raghavan, 2019; Nde, Boldor, Astete, Muley, & Xu, 2016; Yanık, 2017). MAE process induces an increase in temperature and pressure that causes changes in the cell structure, improving the penetration of solvent across the sample matrix.

As per industrial perspective, the optimization of extraction conditions is essential for obtaining the maximum yield of desirable bioactives for any kind of extraction method. The response surface methodology (RSM) for the optimization method is not a new approach but none of the studies has yet been reported regarding optimized extraction protocols for UAE and MAE and their comparative evaluations for extraction of protease enzyme from pineapple waste. The aim of the present study was thus to evaluate the role of extraction techniques, especially MAE and UAEs on the enzymatic activity of the bioactive compounds from pineapple waste. Subsequently, the chemical and bioactive characteristics of the extracts from optimal conditions were also evaluated.

2 | MATERIALS AND METHODS

2.1 | Sampling

The pineapple fruit and wastes (peel, core, and crown) (*Ananus comosus L.*) of Smooth Cayenne cultivars were collected from a local market of Pathum Thani province, Thailand. Each type of pineapple wastes was chopped into pieces (1×1 cm^2) and placed in aluminum trays for 48 hr in an oven at 45°C for drying. The dried samples were finely ground and sieved to obtain particle size (<1 mm). The samples were placed in a plastic bag and stored in a dry dark place at 4°C until further use.

2.2 | Ultrasonic-assisted extraction of pineapple waste

Pineapple peel portion was extracted with distilled water by using various independent factors; ultrasonic amplitude (60–100%), ultrasonication time (10–30 min), and solvent (water) to material ratio (20–40 mL/g) using ultrasonic device (UP200S, 200 W, Hielscher, Teltow, Germany). The solvent temperature was maintained below 10°C during extraction. The extraction mixture was centrifuged (CN-2060, Hsiang Tai Co. Ltd., Taiwan) at $4{,}000 \times g$ for 10 min. The supernatant was further filtered through Whatman No. 1 filter paper (11 μm pore size) and stored at −20°C prior to further use. Finally, the optimized extraction conditions which resulted in high total sugar content, protein content, and proteolytic activity, were used for other pineapple wastes (core and crown).

2.3 | Microwave-assisted extraction of pineapple waste

Pineapple peel portion was extracted with distilled water by using various independent factors; microwave extractor irradiation power

100–300 W, time 5–15 min and feed-to-solvent ratio 1:8–1:12 g/mL based on experimental design, and 2 min of cooling to 30°C. The powder of pineapple wastes (peel portion) was placed in a 250 mL round bottom flask with a measured quantity of distilled water (according to the feed-to-solvent ratio variation). The mixture was irradiated in an enclosed microwave extractor (Sharp-R-3801, Japan). After the extraction process, the mixture was centrifuged (CN-2060, Hsiang Tai Co. Ltd., Taiwan) at 4,000×*g* for 10 min. The supernatant was filtered through Whatman filter paper No. 1 (11 μm) and extracts were stored at −20°C. Finally, the optimized conditions from peel portion were applied to other pineapple wastes (core and crown).

2.4 | Response surface methodology

In the process of UAE and MAE, Box–Behnken design was implemented by using Design Expert 7.0 (Stat-Ease Inc., Minneapolis, MN). The three independent experimental factors for UAE were ultrasonic amplitude (X_1, 60–100%), extraction time (X_2, 10–30 min), and solvent to material ratio (X_3, 20–40 mL/g). Similarly, the three independent experimental factors for MAE were microwave power (X_1, 100–300 W), extraction time (X_2, 5–15 min), and feed-to-solvent ratio (X_3, 1:8–1:12 g/mL). The response variables were yield of total sugar (mg/mL), protein content (mg/mL), and proteolytic activity (U/mL) (Table 1). The influence of independent factors on response variables was evaluated by second-order polynomial model (Equation (1)):

$$Y = A_0 + \sum_{i=1}^{3} A_i X_i + \sum_{i=1}^{3} A_{ii} X_i^2 + \sum_{i=1}^{2} \sum_{j=i+1}^{3} A_{ij} X_i X_j \quad (1)$$

Where Y is the predicted response; A_0 is a constant, A_i is linear coefficient, A_{ii} is quadratic coefficient, and A_{ij} is interaction coefficient. X_i and X_j are coded independent variables.

2.4.1 | Total sugar

Total sugar content was determined by following the method described by Nor, Ramchandran, Duke, and Vasiljevic (2016) with slight modifications. Briefly, the extract (1 mL) was mixed with 1 mL of 5% (v/v) phenol followed by the addition of 5 mL of a concentrated sulfuric acid (98%, v/v). The mixture was allowed to stand for 10 min at 25°C. The mixture was then placed in a water bath for 20 min at 25°C. Color developed was read as absorbance at 480 nm using a UV–Vis spectrophotometer (UNICAM, UV/Vis Spectrophotometer, UK).

2.4.2 | Protein content determination

The total protein content was analyzed by Bradford (1976) assay using bovine serum albumin (BSA) as a reference standard. The extract was centrifuged (CN-2060, Hsiang Tai Co. Ltd., Taiwan) at 4000×*g* for 10 min and 100 μL of the supernatant was added to Bradford reagent and kept for 10 min at 25°C. The absorbance was measured at 595 nm using UV–Vis spectrophotometer (UNICAM, UV/Vis Spectrophotometer, UK) and protein concentration was determined by using BSA standard curve.

TABLE 1 Box–Behnken design with independent variables and analytical responses for UAE

	X_1	X_2	X_3	Analytical responses		
Run	Ultrasonic amplitude (%)	Ultrasonic time (min)	Liquid to material ratio (mL/g)	Total sugar (mg/mL extract)	Total protein (mg/mL extract)	Proteolytic activity (U/mL extract)
1	100	30	30	16.74	3.52	178.51
2	60	10	30	9.03	3.26	172.36
3	80	10	40	4.76	2.97	158.56
4	80	20	30	11.60	3.28	172.81
5	60	20	20	10.43	3.58	182.62
6	80	30	40	6.17	3.19	162.56
7	100	20	20	15.34	3.91	199.77
8	80	20	30	10.28	3.32	174.87
9	80	30	20	12.18	3.74	192.57
10	60	20	40	5.41	3.35	171.31
11	100	10	30	11.40	3.28	174.26
12	100	20	40	6.00	3.19	165.66
13	60	30	30	11.77	3.25	174.91
14	80	20	30	10.46	3.30	174.81
15	80	10	20	11.85	3.58	182.27

2.4.3 | Proteolytic (bromelain) activity determination

The proteolytic activity of the extracts was evaluated by following Nor, Ramchandran, Duke, and Vasiljevic (2015) with slight modifications, using casein and L-tyrosine as a substrate and a standard, respectively. The assay was based on proteolytic hydrolysis of the casein by the enzyme. Bromelain hydrolyzed the casein to release L-tyrosine. One unit of enzyme activity was defined as the amount of enzyme, releasing a product equivalent to 1 μg of tyrosine min^{-1} mL^{-1} under the standard assay conditions and expressed as units per milliliter. The enzyme solution (1 mL) was mixed with 5 mL 0.65% (w/v) of casein, in 0.05 M potassium phosphate buffer (pH 7.5). The reaction was carried out in a water bath at 37°C for 10 min. The reaction was stopped by the addition of 5 mL of 110 mM trichloroacetic acid. The mixture was then filtered through 0.45 μm filter (SC13P045S, HyundaiMicro, Korea). The obtained supernatant was mixed with 5 mL of 500 mM sodium carbonate solution and 1 mL of 20% (v/v) Folin Ciocalteu's phenol reagent and further incubated in water bath at 37°C for 30 min. The solution was filtered through 0.45 μm (SC13P045S, HyundaiMicro, Korea) and the absorbance was read at 660 nm by UV–Vis spectrophotometer (UNICAM, UV/Vis Spectrophotometer, UK).

2.5 | Partial purification of proteins/peptides

The partial purification of enzymes/peptides was conducted by following the method described by Gautam, Mishra, Dash, Goyal, and Rath (2010) with some modifications. Ammonium sulfate (50 g) was added pinch by pinch to the extract (100 mL) and maintained at 5–8°C and the sample was incubated for 45 min with continuous stirring to allow precipitation. The samples were centrifuged (CN-2060, Hsiang Tai Co. Ltd., Taiwan) for 30 min at 4,000×*g*. The supernatant was discarded and the tubes containing pellets were kept in invert direction for 15 min to remove the extra liquid containing salt and contaminants. Then pellets were reconstituted in 0.01 M phosphate buffer (pH 7.0) and used to measure the total protein content and proteolytic activity.

2.6 | Determination of molecular weight

The sample pellets (4 g) were dissolved in 100 mL lysis buffer (pH 8.5 containing 7 M urea, 2 M thiourea) and washed by using 2-D clean-up kit (GE Healthcare, Chicago, IL) followed by reconstitution of protein pellets in a lysis buffer. After measuring the protein concentration (Bio-Rad protein assay, Bio-Rad Laboratory, California, CA), the protein sample (15 μg) was loaded into SDS-polyacrylamide gel (12.5%, w/v) for characterizing the molecular weight of pineapple extracts. Separation of protein was executed at voltage of 15 mA and the gels were stained with colloidal Coomassie blue stain followed by imaging using Image scanner III (GE Healthcare). The molecular weight was estimated by using Bio-Rad's pre-stained broad range molecular standard marker.

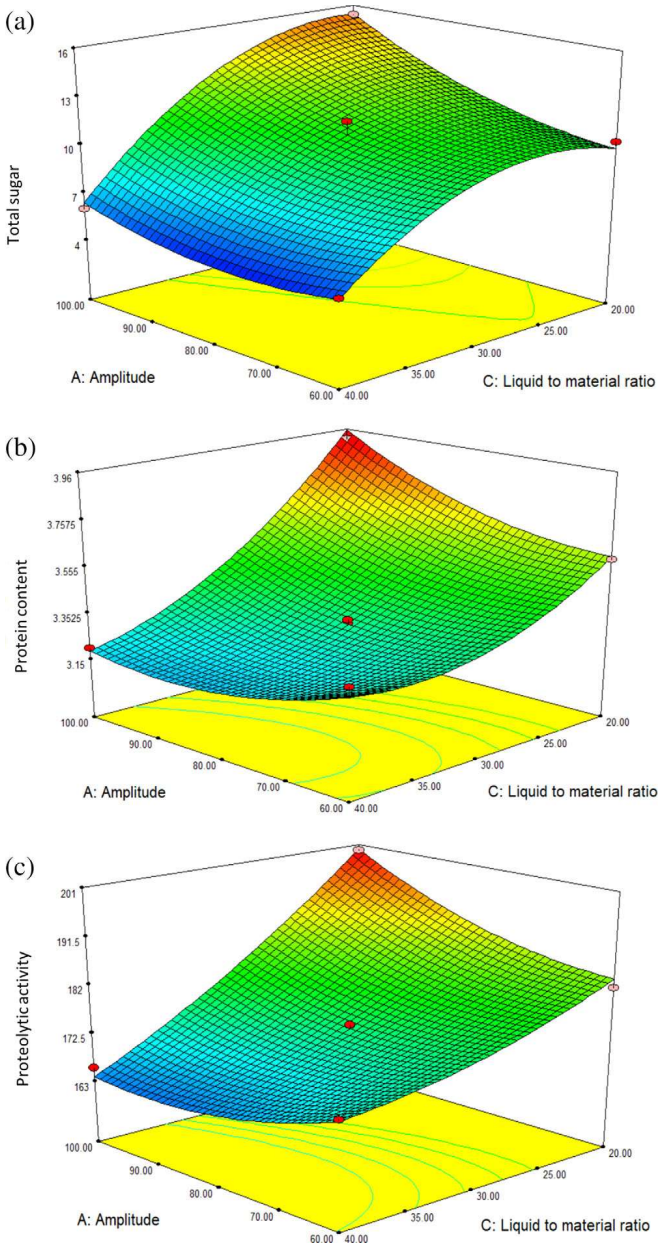

FIGURE 1 Response surface plots of interactive effects of UAE variables on total sugar, protein content, and proteolytic activity

2.7 | Surface morphology of processed pineapple waste

Scanning electron microscope (SEM) was used to evaluate the morphological changes in pineapple wastes samples (core, peel and crown, particle size <1 mm) before and after using UAE and MAE. Samples were dried in freeze dryer and sputtered with a thin layer of gold, and observed under vacuum at accelerating voltage of 5.0 kV at 500× using SEM (SU5000 EM Wizard, Hitachi, Japan).

2.8 | Evaluation of the effect of pH on proteolytic activity

The pH profile of protein extract was determined by evaluating the proteolytic activity in different pH (3–10) conditions. Glycine (pH 3), sodium acetate (pH 4–5), sodium phosphate buffer (pH 6–7), and Tris–HCl buffer (pH 8–10) were used according to Ketnawa et al. (2012) and Júnior et al. (2016) with slight modifications. The extract from pineapple waste was evaluated in terms of relative proteolytic activity. To determine the pH stability, the protein extract was incubated at a given pH for an hour at 25°C and residual protease activity was determined. The relative proteolytic activity was calculated by using the following equation:

$$\text{Relative proteolytic activity}\,(\%) = \frac{\text{Enzyme activity of treated samples x 100}}{\text{Enzyme activity of untreated sample}} \quad (2)$$

2.9 | Evaluation of the effect of temperature on proteolytic activity

Influence of temperature on proteolytic activity of pineapple extracts was performed by exposing them to various temperatures (30, 45, 60, 73, 90, and 95°C) for an hour following the method described by Júnior et al. (2016), with some modifications. The proteolytic activity was determined as described earlier by using casein as a substrate. The relative proteolytic activity was expressed as a percentage of the value recorded with the untreated sample, according to Equation (2).

2.10 | Statistical analysis

The experiments were performed in three replicates and the data were reported as means with SD. Statistical analysis was conducted using a commercial statistical package (SPSS Version 22, Chicago, IL) where ANOVA and Tukey's HSD test were used to determine the significant differences ($p < .05$) among mean observations.

3 | RESULTS AND DISCUSSION

3.1 | Ultrasound-assisted extraction

The total sugar content, protein content, and proteolytic activity of the extracts obtained by UAE from pineapple peel (*Ananus comosus* L.) were in the range of 4.76–16.74 mg/mL, 2.97–3.91 mg/mL, and 158.56–199.77 U/mL, respectively (Table 1). The second order polynomial models were applied to calculate the predicted responses (total sugar, total protein, and proteolytic activity of pineapple peel) as functions of ultrasonic amplitude (X_1), ultrasonic time (X_2), and liquid to material ratio (X_3) as shown in Equations (3)–(5).

$$Y_{\text{Total sugar/UAE}} = 10.79 + 1.60x_1 + 1.23x_2 - 3.43x_3 - 2.49x_3^2 \quad (3)$$

$$Y_{\text{Protein content/UAE}} = 3.30 + 0.057x_1 + 0.077x_2 - 0.27x_3 + 0.064x_1x_2 - 0.12x_1x_3 + 0.084x_1^2 + 0.12x_3^2 \quad (4)$$

$$Y_{\text{Proteolytic activity/UAE}} = 174.16 + 2.12x_1 + 2.64x_2 - 12.39x_3 - 5.70x_1x_3 + 3.35x_1^2 \quad (5)$$

TABLE 2 Box–Behnken design with independent variables and analytical responses for MAE

	X_1	X_2	X_3	Analytical responses		
Run	Microwave power (W)	Irradiation time (min)	Feed to solvent ratio (mL/g)	Total sugar (mg/mL extract)	Total protein (mg/mL extract)	Proteolytic activity (U/mL extract)
1	200	10	10	33.84	1.41	107.03
2	300	10	8	47.69	1.69	102.28
3	300	5	10	36.77	1.51	102.28
4	200	10	10	32.22	1.34	92.78
5	300	10	12	47.50	1.33	118.12
6	200	15	8	37.32	1.69	103.86
7	100	5	10	29.90	2.55	164.04
8	200	10	10	33.35	1.62	124.45
9	200	5	8	31.64	2.07	126.03
10	200	15	12	35.39	1.33	122.87
11	300	15	10	49.58	1.44	110.20
12	200	5	12	31.28	1.33	124.45
13	100	15	10	33.66	1.72	154.54
14	100	10	12	33.03	1.86	198.88
15	100	10	8	33.19	2.43	160.87

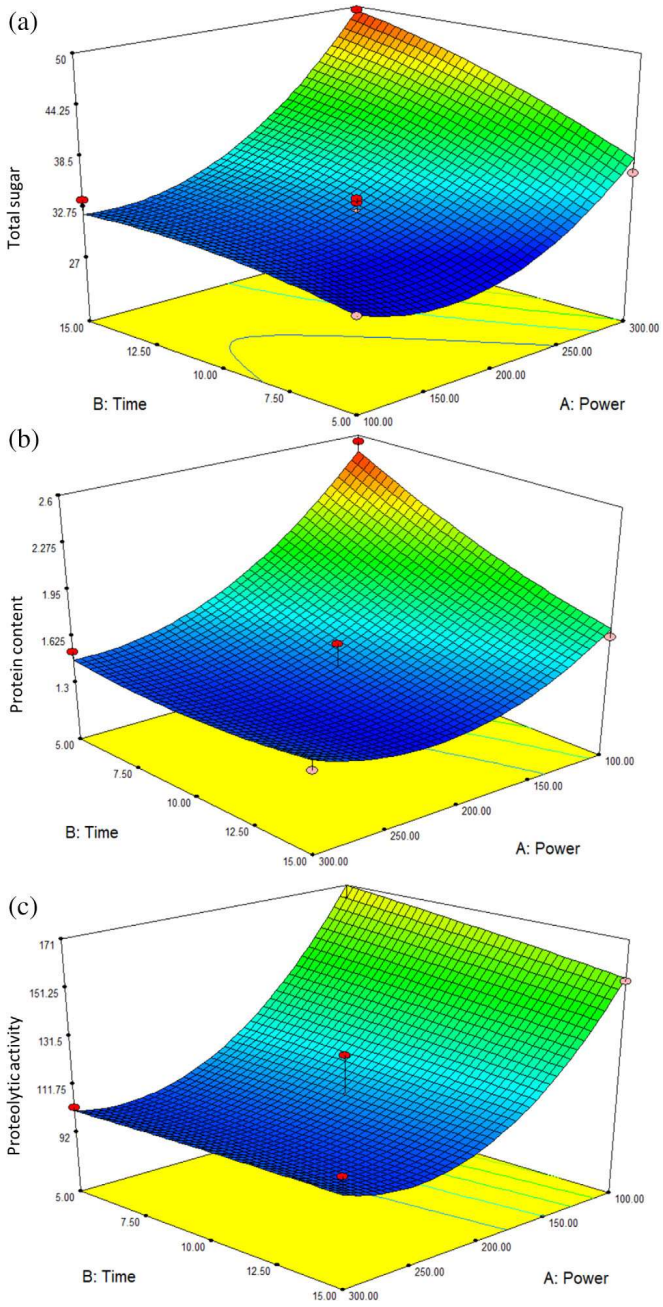

FIGURE 2 Response surface plots of interactive effects of MAE variables on total sugar, protein content, and proteolytic activity

The applied regression models were significant ($p < .05$) for total sugar content, protein content, and proteolytic activity. All the independent variables significantly ($p < .05$) influenced the response variables. For total sugar, protein content, and proteolytic activity, coefficient of correlation (R^2) values were 0.960, 0.986, and 0.985, respectively (Supporting Information, Table S1). Lack of fit was insignificant which indicated that models were well fitted to experimental data.

Figure 1 illustrates the response surface plots for the interactive effects of extraction parameters (ultrasonic amplitude, ultrasonic time, and liquid to material ratio). The total sugar, protein content, and proteolytic activity increased with the increasing ultrasonic amplitude. In ultrasonic treatment, the high frequency sound waves generate cavitation, mechanical force and thermal impact which increase the mass transfer by disrupting the cell walls and increase the rate of diffusion into the solvent (Belwal et al., 2018; Wang, Liang, & Yuan, 2011). The shear effect generated due to mechanical forces rupture the plant cell wall and thus, increases the extraction efficiency during ultrasonication (Sharmila et al., 2016; Zeković et al., 2017). Moreover, higher ultrasonic power increases the extraction temperature and finally leads to the solubility of analytes in the solvent making it easier for diffusion from the sample matrix to the outer region (da Rosa et al., 2019; Esclapez, García-Pérez, Mulet, & Cárcel, 2011).

Solvent to material ratio is an important factor in the optimization of UAE (Hadidi et al., 2020). Total sugar, protein content and proteolytic activity decreased when the liquid to material ratio increased from 20 to 40 mL/g. As the ratio of liquid to material was increased markedly, the ultrasonic energy associated with the unit volume decreases, resulting in decrease of extraction yield (Szydłowska-Czerniak, Dianoczki, Recseg, Karlovits, & Szłyk, 2008). However, an increase in the liquid to material ratio enhanced the osmotic pressure and contact area, resulting in more solvent being pushed into the plant matrix, and enhancing the penetration of bioactive chemicals through the cell wall into the solvent (Prasad, Yang, Yi, Zhao, & Jiang, 2009).

3.2 | Microwave-assisted extraction

The total sugar, protein content, and proteolytic activity of the pineapple peel extract obtained by MAE were in the range of 29.90–49.58 mg/mL, 1.33–2.55 mg/mL, and 92.78–198.88 U/mL extract, respectively (Table 2). The second order polynomial models were applied to calculate the predicted responses (total sugar, protein content, and proteolytic activity of pineapple peel) as a function of independent MAE parameters; microwave power (X_1), irradiation time (X_2), and feed to solvent ratio (X_3) (Equations (6)–(8)).

$$Y_{\text{Total sugar/MAE}} = 33.14 + 6.47x_1 + 3.29x_2 + 2.26x_1x_2 + 5.39x_1^2 \quad (6)$$

$$Y_{\text{Protein content/MAE}} = 1.46 - 0.32x_1 - 0.16x_2 - 0.25x_3 + 0.19x_1x_2 + 0.29x_1^2 \quad (7)$$

$$Y_{\text{Proteolytic activity/MAE}} = 108.09 - 30.68x_1 + 25.20x_1^2 \quad (8)$$

The applied regression models were significant ($p < .05$) for total sugar, protein content, and proteolytic activity. All the independent variables significantly ($p < .05$) influenced the response variables. For total sugar, protein content, and proteolytic activity, coefficient of correlation (R^2) values were 0.979, 0.962, and 0.929, respectively (Supporting Information, Table S2).

The interactive effects of MAE independent extraction parameters (microwave power, irradiation time, and feed to solvent ratio) on response variables were presented by response surface plots

TABLE 3 Total sugars, protein content and proteolytic activity of optimized pineapple peel, core and crown extracts

	UAE			MAE		
Pineapple byproducts	Total sugar (mg/mL extract)	Protein content (mg/mL extract)	Proteolytic activity (U/mL extract)	Total sugar (mg/mL extract)	Protein content (mg/mL extract)	Proteolytic activity (U/mL extract)
Core	32.55 ± 0.02[a]	1.73 ± 0.08[c]	120.45 ± 3.29[c]	83.76 ± 0.06[a]	0.47 ± 0.01[c]	51.29 ± 2.59[c]
Peel	15.71 ± 0.03[b]	4.01 ± 0.04[b]	196.46 ± 3.29[b]	33.87 ± 0.03[b]	2.50 ± 0.01[b]	154.08 ± 1.49[b]
Crown	6.20 ± 0.01[c]	4.75 ± 0.05[a]	289.57 ± 5.70[a]	13.41 ± 0.03[c]	3.47 ± 0.01[a]	224.91 ± 2.59[a]

Note: Superscript (a–c) letters within column indicate significant differences between mean observations.

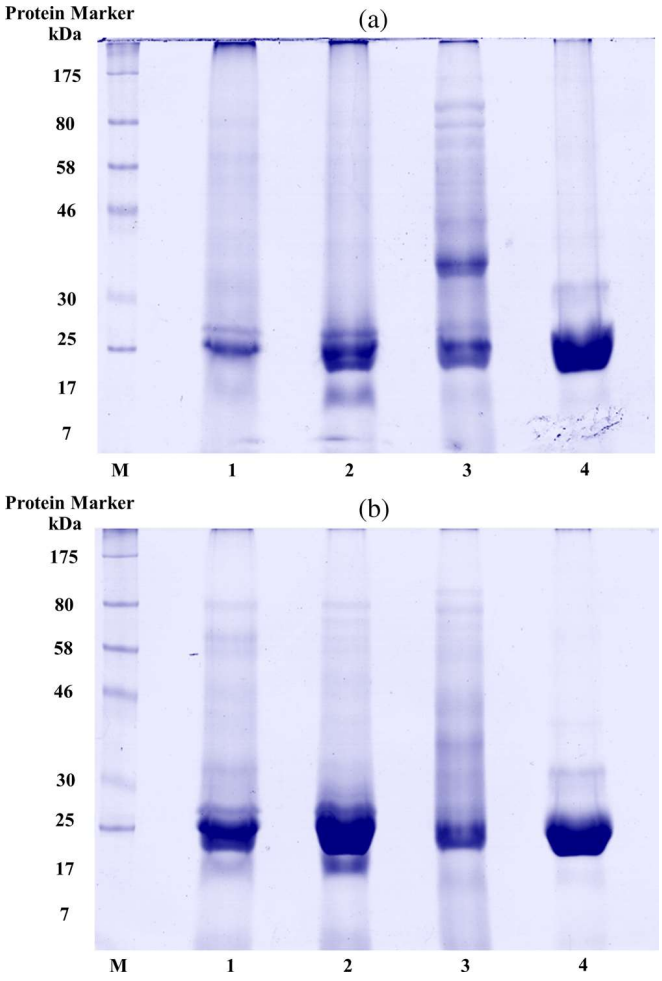

FIGURE 3 Protein patterns of enzyme extracts after UAE (a) and MAE (b) from pineapple wastes. M = molecular weight marker (kDa); lanes 1 = core; lanes 2 = peel; lanes 3 = crown; lanes 4 = pineapple pulp

(Figure 2). The microwave power had significant effect ($p < .05$) on total sugar, protein content, and proteolytic activity. The interactive effect of microwave power and irradiation time had significant effect on total sugar and protein content; however, none of interaction terms had significant effect on proteolytic activity.

The total sugar content increased with increasing microwave power until the power reached to 300 W. During the microwave treatment, the energy of microwave radiation rapidly transferred to plant material and solvent through ionic conduction and dipolar rotation, which swiftly warmed plant cells (Moreira et al., 2017; Yan et al., 2010), and increase pressure inside the cells and accelerated the destruction of cell wall matrix and epidermal tissue (Rodsamran & Sothornvit, 2019). This phenomenon led to permeation channel for intracellular plant solutes to enter the periphery solvent thereby contributing to the rapid dissolution of solute and high yield of oligosaccharides (Zhang, Yang, & Liu, 2008). On the other hand, it was observed that the protein content and proteolytic activity were decreased with increase in microwave power from 100 to 300 W and increase in irradiation time. It could be explained by the fact that, high power microwave irradiation can degrade the thermolabile bioactive compounds and decrease their yield (Dahmoune et al., 2014; Mandal & Mandal, 2010).

3.3 | Optimization of UAE and MAE conditions

UAE optimal extraction conditions were determined by desirability function of Design Expert as ultrasonic amplitude 99.96%, extraction time 26.83 min, and liquid to material ratio 20.96 mL/g. At this condition, the total sugar, protein content, and proteolytic activity were predicted as 16.77 mg/mL, 4.01 mg/mL, and 201.85 U/mL, respectively. At optimized UAE of pineapple peel, the experimental values for the total sugar, protein content, and proteolytic activity were 15.71 ± 0.03 mg/mL, 4.01 ± 0.04 mg/mL, and 196.46 ± 3.29 U/mL, respectively.

The optimal conditions for MAE were determined by using desirability function of Design Expert as microwave power 100 W, irradiation time 8.99 min, and feed to solvent ratio 1:8 g/mL. At this condition, the total sugar, protein content, and proteolytic activity from pineapple peel were predicted as 33.87 mg/mL, 2.55 mg/mL, and 163.81 U/mL, respectively. At optimized MAE of peels, the experimental values for total sugar, protein content, and proteolytic activity were 33.87 ± 0.03 mg/mL, 2.50 ± 0.01 mg/mL, and 154.08 ± 1.49 U/mL, which were close to the predicted values and confirm the validity of the optimized model. The extraction of pineapple core and crown was also carried out at optimized extraction conditions (for peels) and total sugars, protein content, and proteolytic activity of the extracts are summarized in Table 3.

The core of pineapple showed the highest total sugars contents with the values of 32.55 and 83.76 mg/mL extract in UAE and MAE, respectively, whereas pineapple crown was found with lowest total

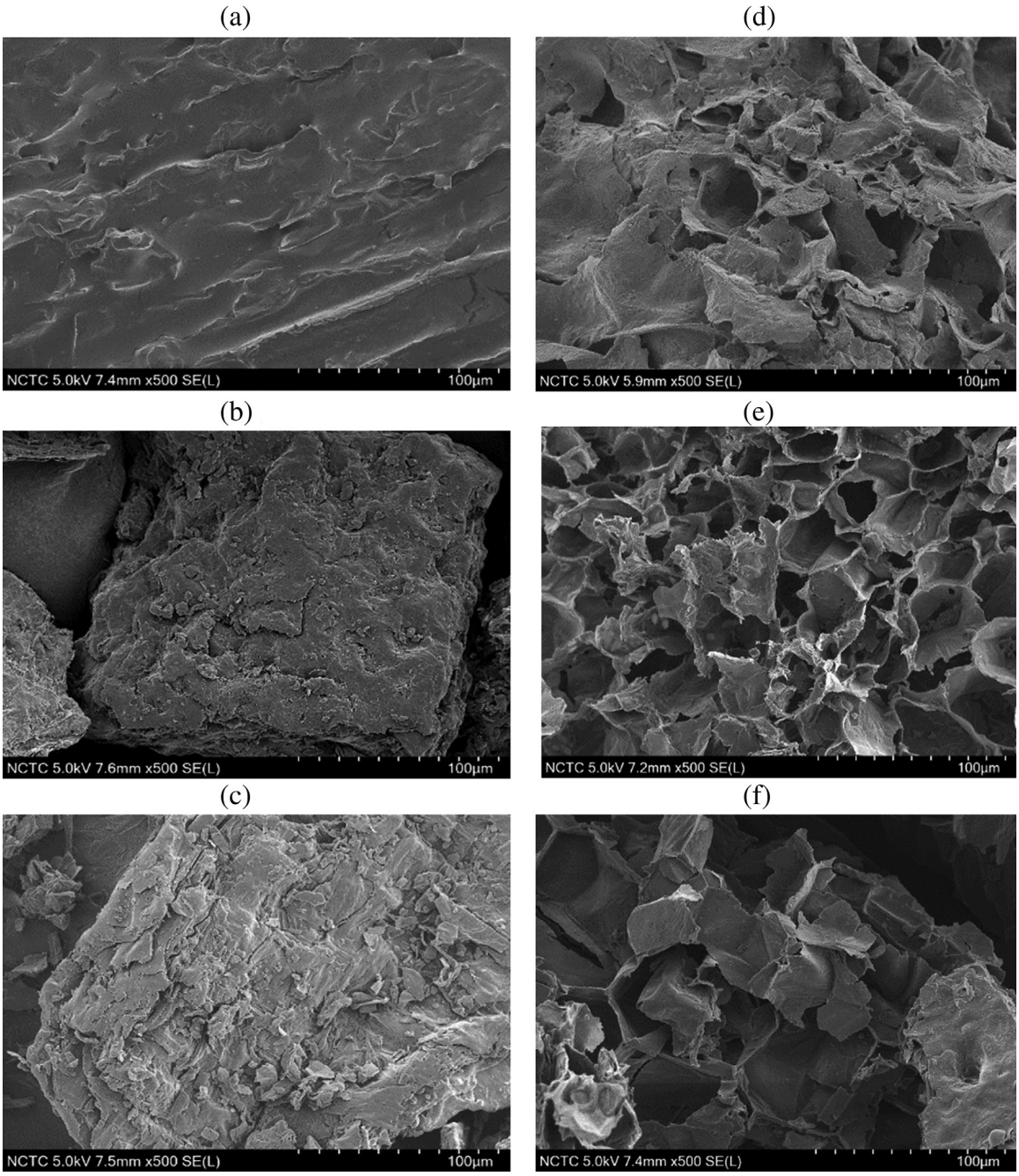

FIGURE 4 SEM images (a–f, ×500) of pineapple wastes before ultrasonic extraction (a–c) and, after ultrasonic extraction (d–f) at optimized condition: Untreated core (a); untreated peel (b); untreated crown (c); treated core (d); treated peel (e); treated crown (f)

sugar contents of 6.20 and 13.41 mg/mL extract in UAE and MAE, respectively. The total soluble content in terms of °Brix value was reported to be high in pineapple core (4.73–6.27 °Brix) as compared to peel (4.33–4.43 °Brix) and crown (3–3.43 °Brix) in Nang Lae and Phu Lae pineapple, respectively (Ketnawa et al., 2012). The results showed that at optimum condition MAE provided higher total sugar content than UAE. During MAE, increasing temperature resulted in increases in intra-cellular pressure which causes the cell rupture, thereby increased the extraction rates (da Rosa et al., 2019; Khajeh, Akbari Moghaddam, & Sanchooli, 2010; Rafiee, Jafari, Alami, & Khomeiri, 2011).

The extract from the pineapple crown exhibited significantly higher total protein contents in UAE and MAE (4.75 and 3.47 mg/mL extract, respectively) and proteolytic activity (289.57 and 224.91 U/mL extract, respectively) in comparison to other pineapple byproducts. Silvestre et al. (2012) reported that protein content of 2.2–3.1 mg/mL and proteolytic activity of 6.7–10.6 U/mL in crude extract of pineapple peel after precipitation with different concentrations of ammonium sulfate (40–80% w/v).

MAE extraction resulted in high total sugar contents in short extraction time, whereas UAE provided higher values for protein content and proteolytic activity than the MAE. MAE resulted in low

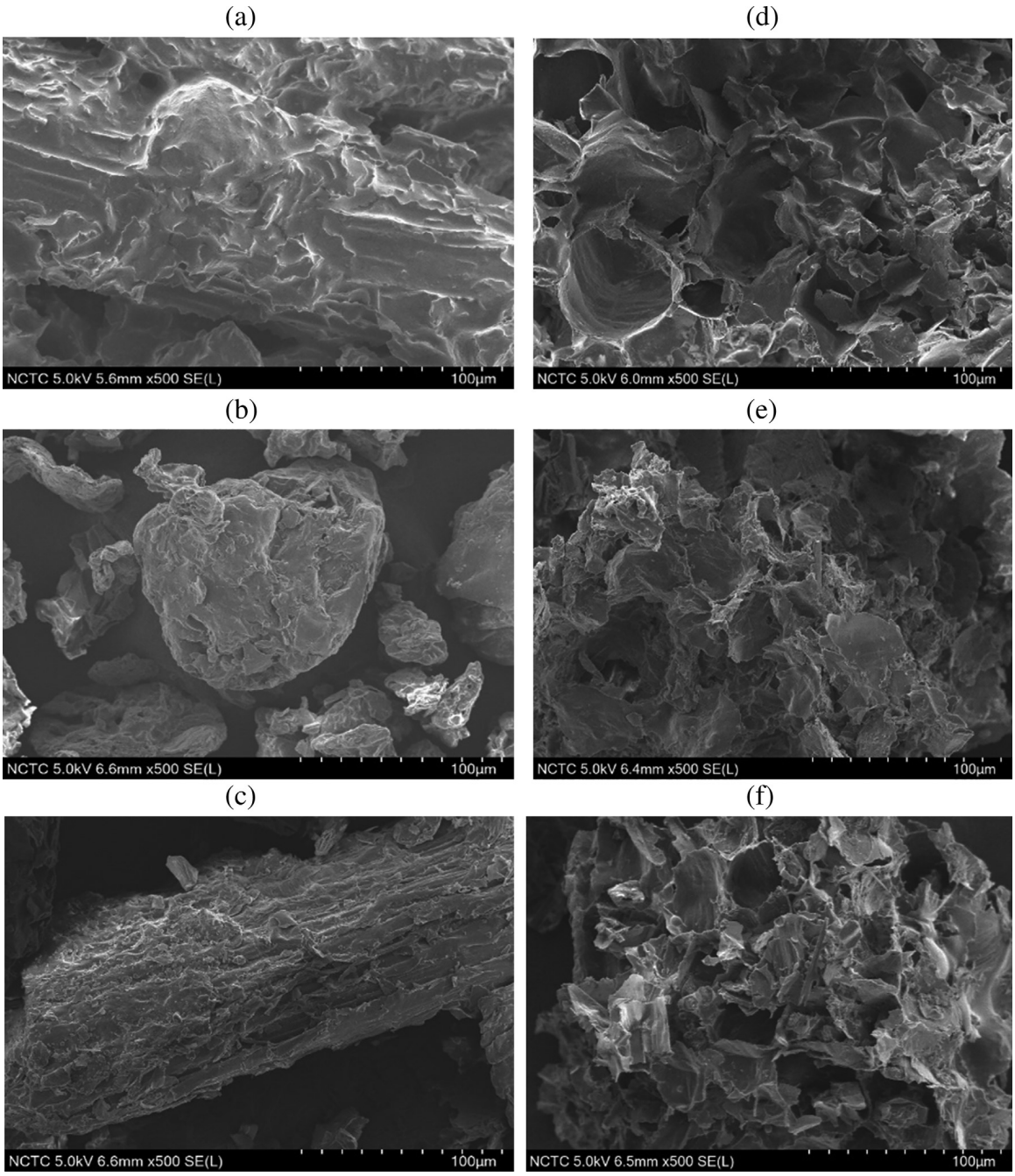

FIGURE 5 SEM images (a–f, ×500) of pineapple wastes before microwave extraction (a–c), and after microwave extraction (d–f) at optimized condition: Untreated core (a); untreated peel (b); untreated crown (c); treated core (d); treated peel (e); treated crown (f)

protein content and proteolytic activity, due to high temperature treatment associated with MAE, which resulted in degradation of thermolabile proteins (Dahmoune et al., 2014).

3.4 | Characterization of protein components by SDS-PAGE

Figure 3 illustrates the diffused bands of the main protein components with the molecular weight of about ~17, 23–28, and 39 kDa. The molecular weight of bromelain is about 23–26 kDa depending on the parts (stems, fruits, core, peels) of pineapple (Maurer, 2001; Umesh Hebbar, Sumana, & Raghavarao, 2008). Moreover, Ketnawa et al. (2012) reported that the bromelain extracted from pineapple stems and crowns showed high intensity protein band around ~28 kDa. Similarly, the main protein components obtained from pineapple byproducts by MAE showed bands at MW ~17, and 20–26 kDa. Plant-based bioactive peptides have been reported to prevent metabolic disorders and lifestyle-related diseases (Mazorra-Manzano, Ramírez-Suarez, & Yada, 2018). Bromelain enzyme was

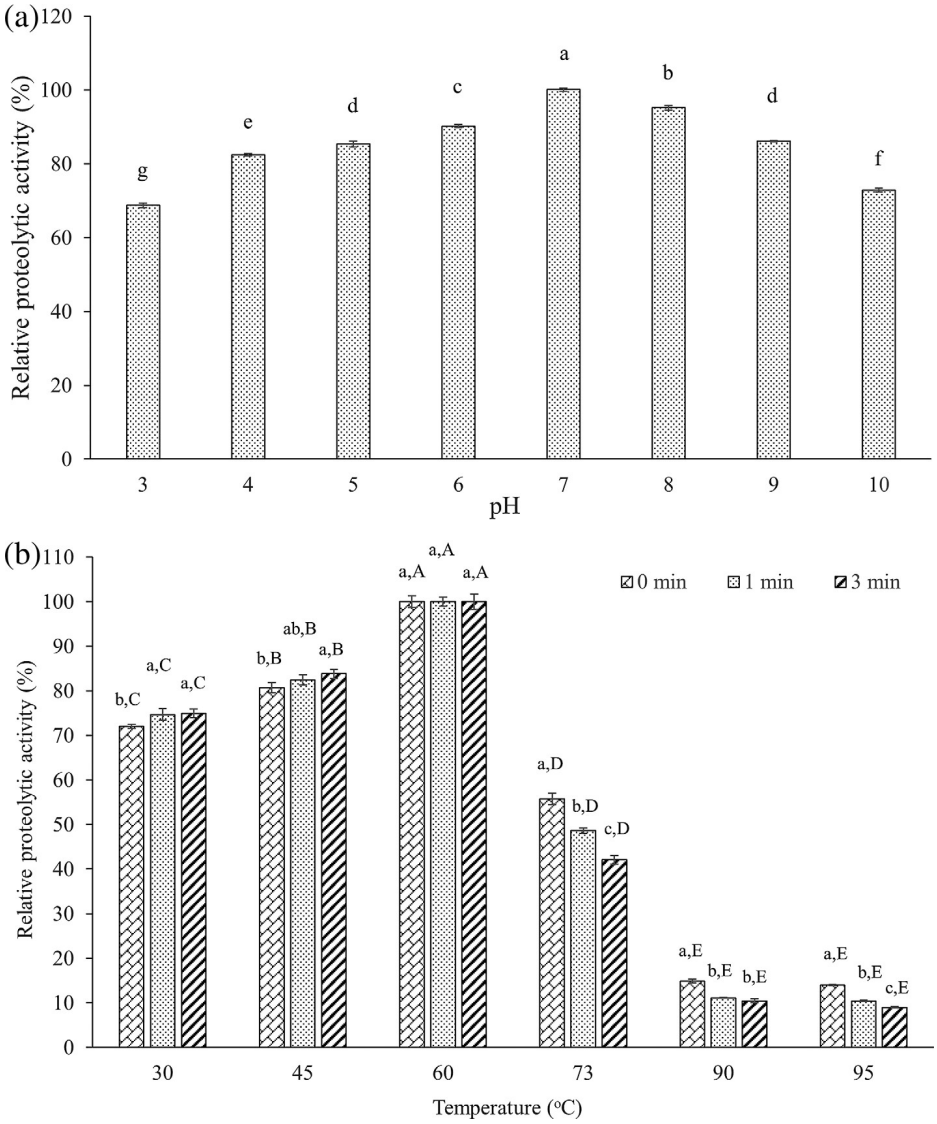

FIGURE 6 Effect of pH (a) and temperature (b) on proteolytic activity of pineapple crown partially purified proteins. Different superscript letters above the bars indicate significant differences ($p < .05$) among mean observations

reported to reduce the allergenicity in raw peanuts (Yu & Mikiashvili, 2020).

3.5 | Surface morphology of processed pineapple waste

The structure and surface morphology of the pineapple byproducts, before and after the extraction are presented in Figures 4 and 5. Compared with UAE, MAE had a more drastic effect on the cell walls of the plant tissues and it is due to high temperature and intracellular pressure associated with MAE (Cheng et al., 2015; Wang et al., 2009). After MAE treatment sample surface became disorganized and ruptured completely, with irregular pores and cavities (Figure 5). Microwave irradiation made a rapid temperature increase in a short time within the cellular structures, which led to the rupture of cellular structures to facilitate the rapid release of target substances (Hu et al., 2018). Pineapple byproducts after UAE treatment showed disruptions on surface (Figure 4) but the cell surfaces were partly affected as compared to MAE treatment (Figure 5). Disintegration patterns in samples were different after UAE and MAE. The high yield of target compounds after UAE and MAE methods is attributed to the disruption of cellular structure followed by better penetration of solvent inside the matrix with acoustic cavitation/enhanced temperature (Sarkar et al., 2021).

3.6 | Effect of pH and temperature on proteolytic activity

The proteolytic activity of pineapple crown was significantly higher than the peel and core, therefore the influence of pH and temperature on proteolytic activity of crown proteins was evaluated. The effect of pH (3–10) on proteolytic activity of the crown extract was measured and reported as a relative proteolytic activity against the control (Figure 6a). The extract from pineapple crown exhibited a variable pH activity profile and a high proteolytic activity was observed within a pH range of 6–8. The maximum proteolytic activity was found at

neutral around pH 7. Silvestre et al. (2012) also reported that the proteolytic enzymes from the pineapple peel (*Ananas comosus*) exhibited high enzymatic activity at pH values of 6.0 and 7.0. The activity of bromelain was markedly decreased at high acidic (pH 3–4) and alkaline conditions (pH 9–10). Xue et al. (2010) reported optimum pH range for stem bromelain as 6–7. While the optimum pH range for bromelain from pineapple peel was reported at pH 6 for crude enzymatic extract and 7 for precipitated enzymatic extract. (Silvestre et al., 2012).

Effect of temperature on proteolytic activity of crown extract was evaluated in the range of 30–95°C for 0–3 min. As the incubation temperature was increased, the relative proteolytic activity also increased and reached the highest point at 60°C followed by a decrease with further increase in temperature (Figure 6b). The optimum temperature was reported to be 63°C for the proteolytic enzymes from ripe fruits of *B. antiacantha* Bertol (Bromeliaceae) (Vallés, Furtado, & Cantera, 2007). Koh, Kang, Kim, Cha, and Kwon (2006) also reported that the enzyme activity of pineapple produced in jeju-island was optimal at 60°C. At each incubation temperature from 30 to 60°C, relative proteolytic activity (%) was increased as the incubation time increased from 0 to 3 min and at the high temperature (73–95°C), the relative proteolytic activity from 0 to 3 min was decreased.

4 | CONCLUSIONS

Pineapple byproducts are good source of bioactive peptides and functional proteins. Optimized extraction by UAE and MAE indicated that the high yield of proteins and good proteolytic activity can be obtained within a short period of time. However, UAE of pineapple byproducts presented high proteolytic activity which was associated with the UAE operation at low temperature. Among pineapple byproducts, crown presented significantly high protein yield and proteolytic activity. Bromelain and bioactive peptides extracted from pineapple byproducts can be used for food and feed product development.

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AUTHOR CONTRIBUTIONS

Thatchajaree Mala: Conceptualization; formal analysis; methodology; validation; writing-original draft. **Muhammad Bilal Sadiq:** Conceptualization; formal analysis; methodology; writing-original draft. **Anil Anal:** Conceptualization; supervision; visualization; writing-original draft.

CONFLICT OF INTEREST

The authors declare no potential conflict of interest.

DATA AVAILABILITY STATEMENT

Data will be available on request from corresponding author.

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REFERENCES

Barba, F. J., Galanakis, C. M., Esteve, M. J., Frigola, A., & Vorobiev, E. (2015). Potential use of pulsed electric technologies and ultrasounds to improve the recovery of high-added value compounds from blackberries. *Journal of Food Engineering*, *167*, 38–44.

Belwal, T., Ezzat, S. M., Rastrelli, L., Bhatt, I. D., Daglia, M., Baldi, A., … Atanasov, A. G. (2018). A critical analysis of extraction techniques used for botanicals: Trends, priorities, industrial uses and optimization strategies. *TrAC–Trends in Analytical Chemistry*, *100*, 82–102. https://doi.org/10.1016/j.trac.2017.12.018

Bradford, M. M. (1976). A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Analytical Biochemistry*, *72*(1–2), 248–254. https://doi.org/10.1016/0003-2697(76)90527-3

Cheng, Z., Song, H., Yang, Y., Liu, Y., Liu, Z., Hu, H., & Zhang, Y. (2015). Optimization of microwave-assisted enzymatic extraction of polysaccharides from the fruit of *Schisandra chinensis* Baill. *International Journal of Biological Macromolecules*, *76*, 161–168. https://doi.org/10.1016/j.ijbiomac.2015.01.048

da Rosa, G. S., Vanga, S. K., Gariepy, Y., & Raghavan, V. (2019). Comparison of microwave, ultrasonic and conventional techniques for extraction of bioactive compounds from olive leaves (*Olea europaea* L.). *Innovative Food Science and Emerging Technologies*, *58*. https://doi.org/10.1016/j.ifset.2019.102234

Dahmoune, F., Spigno, G., Moussi, K., Remini, H., Cherbal, A., & Madani, K. (2014). *Pistacia lentiscus* leaves as a source of phenolic compounds: Microwave-assisted extraction optimized and compared with ultrasound-assisted and conventional solvent extraction. *Industrial Crops and Products*, *61*, 31–40. https://doi.org/10.1016/j.indcrop.2014.06.035

Esclapez, M. D., García-Pérez, J. V., Mulet, A., & Cárcel, J. A. (2011). Ultrasound-assisted extraction of natural products. *Food Engineering Reviews*, *3*(2), 108–120. https://doi.org/10.1007/s12393-011-9036-6

FAOSTAT. (2020). *Production of pineapples: Top 10 producers*. FAO: Rome, Italy. http://www.fao.org/faostat/en/#data/QC/visualize

Feijoo-Siota, L., & Villa, T. G. (2011). Native and biotechnologically engineered plant proteases with industrial applications. *Food and Bioprocess Technology*, *4*(6), 1066–1088. https://doi.org/10.1007/s11947-010-0431-4

Galanakis, C. M. (2015). Separation of functional macromolecules and micromolecules: From ultrafiltration to the border of nanofiltration. *Trends in Food Science & Technology*, *42*(1), 44–63.

Galanakis, C. M. (2020). The food systems in the era of the coronavirus (COVID-19) pandemic crisis. *Food*, *9*(4), 523.

Galanakis, C. M., Aldawoud, T., Rizou, M., Rowan, N. J., & Ibrahim, S. A. (2020). Food ingredients and active compounds against the coronavirus disease (COVID-19) pandemic: A comprehensive review. *Food*, *9*(11), 1701.

Galanakis, C. M., Rizou, M., Aldawoud, T. M., Ucak, I., & Rowan, N. J. (2021). Innovations and technology disruptions in the food sector within the COVID-19 pandemic and post-lockdown era. *Trends in Food Science & Technology*, *110*, 193–200.

Galanakis, C. M. (2021). Functionality of food components and emerging technologies. *Food*, *10*(1), 128.

Gautam, S. S., Mishra, S. K., Dash, V., Goyal, A. K., & Rath, G. (2010). Comparative study of extraction, purification and estimation of bromelain from stem and fruit of pineapple plant. *Thai Journal of Pharmaceutical Sciences*, *34*(2), 67–76.

Grzonka, Z., Kasprzykowski, F., & Wiczk, W. (2007). Cysteine proteases. *Industrial Enzymes: Structure, Function and Applications*, 181–195. Springer: New York, NY. https://doi.org/10.1007/1-4020-5377-0_11

Hadidi, M., Amoli, P. I., Jelyani, A. Z., Hasiri, Z., Rouhafza, A., Ibarz, A., … Tabrizi, S. T. (2020). Polysaccharides from pineapple core as a canning by-product: Extraction optimization, chemical structure, antioxidant and functional properties. *International Journal of Biological Macromolecules*, *163*, 2357–2364.

Hu, B., Zhou, K., Liu, Y., Liu, A., Zhang, Q., Han, G., … Zhu, D. (2018). Optimization of microwave-assisted extraction of oil from tiger nut (*Cyperus esculentus* L.) and its quality evaluation. *Industrial Crops and Products*, *115*, 290–297. https://doi.org/10.1016/j.indcrop.2018.02.034

Jain, S., & Anal, A. K. (2016). Optimization of extraction of functional protein hydrolysates from chicken egg shell membrane (ESM) by ultrasonic assisted extraction (UAE) and enzymatic hydrolysis. *LWT—Food Science and Technology*, *69*, 295–302. https://doi.org/10.1016/j.lwt.2016.01.057

Ketnawa, S., Chaiwut, P., & Rawdkuen, S. (2012). Pineapple wastes: A potential source for bromelain extraction. *Food and Bioproducts Processing*, *90*(3), 385–391. https://doi.org/10.1016/j.fbp.2011.12.006

Ketnawa, S., Rawdkuen, S., & Chaiwut, P. (2010). Two phase partitioning and collagen hydrolysis of bromelain from pineapple peel Nang Lae cultivar. *Biochemical Engineering Journal*, *52*(2–3), 205–211. https://doi.org/10.1016/j.bej.2010.08.012

Khajeh, M., Akbari Moghaddam, A. R., & Sanchooli, E. (2010). Application of Doehlert design in the optimization of microwave-assisted extraction for determination of zinc and copper in cereal samples using FAAS. *Food Analytical Methods*, *3*(3), 133–137. https://doi.org/10.1007/s12161-009-9099-7

Koh, J., Kang, S. M., Kim, S. J., Cha, M. K., & Kwon, Y. J. (2006). Effect of pineapple protease on the characteristics of protein fibers. *Fibers and Polymers*, *7*(2), 180–185. https://doi.org/10.1007/BF02908264

Koirala, S., Prathumpai, W., & Anal, A. K. (2021). Effect of ultrasonication pretreatment followed by enzymatic hydrolysis of caprine milk proteins and on antioxidant and angiotensin converting enzyme (ACE) inhibitory activity of peptides thus produced. *International Dairy Journal*, *118*, 105026.

Júnior, L., B. R, d. C., Tribst, A. A. L., Bonafe, C. F. S., & Cristianini, M. (2016). Determination of the influence of high pressure processing on calf rennet using response surface methodology: Effects on milk coagulation. *LWT—Food Science and Technology*, *65*, 10–17. https://doi.org/10.1016/j.lwt.2015.07.063

Mandal, V., & Mandal, S. C. (2010). Design and performance evaluation of a microwave based low carbon yielding extraction technique for naturally occurring bioactive triterpenoid: Oleanolic acid. *Biochemical Engineering Journal*, *50*(1–2), 63–70. https://doi.org/10.1016/j.bej.2010.03.005

Mazorra-Manzano, M. A., Ramírez-Suarez, J. C., & Yada, R. Y. (2018). Plant proteases for bioactive peptides release: A review. *Critical Reviews in Food Science and Nutrition*, *58*(13), 2147–2163.

Maurer, H. R. (2001). Bromelain: Biochemistry, pharmacology and medical use. *Cellular and Molecular Life Sciences*, *58*(9), 1234–1245. https://doi.org/10.1007/PL00000936

Moreira, M. M., Barroso, M. F., Boeykens, A., Withouck, H., Morais, S., & Delerue-Matos, C. (2017). Valorization of apple tree wood residues by polyphenols extraction: Comparison between conventional and microwave-assisted extraction. *Industrial Crops and Products*, *104*, 210–220. https://doi.org/10.1016/j.indcrop.2017.04.038

Nakthong, N., Wongsagonsup, R., & Amornsakchai, T. (2017). Characteristics and potential utilizations of starch from pineapple stem waste. *Industrial Crops and Products*, *105*, 74–82. https://doi.org/10.1016/j.indcrop.2017.04.048

Nde, D. B., Boldor, D., Astete, C., Muley, P., & Xu, Z. (2016). Oil extraction from sheanut (*Vitellaria paradoxa* Gaertn C.F.) kernels assisted by microwaves. *Journal of Food Science and Technology*, *53*(3), 1424–1434. https://doi.org/10.1007/s13197-015-2160-1

Nor, M. Z. M., Ramchandran, L., Duke, M., & Vasiljevic, T. (2015). Characteristic properties of crude pineapple waste extract for bromelain purification by membrane processing. *Journal of Food Science and Technology*, *52*(11), 7103–7112. https://doi.org/10.1007/s13197-015-1812-5

Nor, M. Z. M., Ramchandran, L., Duke, M., & Vasiljevic, T. (2016). Separation of bromelain from crude pineapple waste mixture by a two-stage ceramic ultrafiltration process. *Food and Bioproducts Processing*, *98*, 142–150. https://doi.org/10.1016/j.fbp.2016.01.001

Pimentel-Moral, S., Borrás-Linares, I., Lozano-Sánchez, J., Arráez-Román, D., Martínez-Férez, A., & Segura-Carretero, A. (2018). Microwave-assisted extraction for *Hibiscus sabdariffa* bioactive compounds. *Journal of Pharmaceutical and Biomedical Analysis*, *156*, 313–322. https://doi.org/10.1016/j.jpba.2018.04.050

Prasad, K. N., Yang, E., Yi, C., Zhao, M., & Jiang, Y. (2009). Effects of high pressure extraction on the extraction yield, total phenolic content and antioxidant activity of Longan fruit pericarp. *Innovative Food Science and Emerging Technologies*, *10*(2), 155–159. https://doi.org/10.1016/j.ifset.2008.11.007

Rafiee, Z., Jafari, S. M., Alami, M., & Khomeiri, M. (2011). Microwave-assisted extraction of phenolic compounds from olive leaves: A comparison with maceration. *Journal of Animal and Plant Sciences*, *21*(4), 738–745.

Rodsamran, P., & Sothornvit, R. (2019). Extraction of phenolic compounds from lime peel waste using ultrasonic-assisted and microwave-assisted extractions. *Food Bioscience*, *28*, 66–73. https://doi.org/10.1016/j.fbio.2019.01.017

Saenmuangchin, R., & Siripinyanond, A. (2019). Identification of white scale formation in pineapple juice concentrate. *Microchemical Journal*, *147*, 1180–1185. https://doi.org/10.1016/j.microc.2019.04.054

Sarkar, R., Bhowmik, A., Kundu, A., Dutta, A., Nain, L., Chawla, G., & Saha, S. (2021). Inulin from *Pachyrhizus erosus* root and its production intensification using evolutionary algorithm approach and response surface methodology. *Carbohydrate Polymers*, *251*, 1–11. https://doi.org/10.1016/j.carbpol.2020.117042

Sepúlveda, L., Romaní, A., Aguilar, C. N., & Teixeira, J. (2018). Valorization of pineapple waste for the extraction of bioactive compounds and glycosides using autohydrolysis. *Innovative Food Science & Emerging Technologies*, *47*, 38–45.

Sharmila, G., Nikitha, V. S., Ilaiyarasi, S., Dhivya, K., Rajasekar, V., Kumar, N. M., … Muthukumaran, C. (2016). Ultrasound assisted extraction of total phenolics from *Cassia auriculata* leaves and evaluation of its antioxidant activities. *Industrial Crops and Products*, *84*, 13–21. https://doi.org/10.1016/j.indcrop.2016.01.010

Silvestre, M. P. C., Carreira, R. L., Silva, M. R., Corgosinho, F. C., Monteiro, M. R. P., & Morais, H. A. (2012). Effect of pH and temperature on the activity of enzymatic extracts from pineapple peel. *Food and Bioprocess Technology*, *5*(5), 1824–1831. https://doi.org/10.1007/s11947-011-0616-5

Szydłowska-Czerniak, A., Dianoczki, C., Recseg, K., Karlovits, G., & Szłyk, E. (2008). Determination of antioxidant capacities of vegetable oils by ferric-ion spectrophotometric methods. *Talanta*, *76*(4), 899–905. https://doi.org/10.1016/j.talanta.2008.04.055

Umesh Hebbar, H., Sumana, B., & Raghavarao, K. S. M. S. (2008). Use of reverse micellar systems for the extraction and purification of bromelain from pineapple wastes. *Bioresource Technology*, *99*(11), 4896–4902. https://doi.org/10.1016/j.biortech.2007.09.038

Vallés, D., Furtado, S., & Cantera, A. M. B. (2007). Characterization of news proteolytic enzymes from ripe fruits of *Bromelia antiacantha* Bertol. (Bromeliaceae). *Enzyme and Microbial Technology*, *40*(3), 409–413. https://doi.org/10.1016/j.enzmictec.2006.07.011

Wang, J., Zhang, J., Wang, X., Zhao, B., Wu, Y., & Yao, J. (2009). A comparison study on microwave-assisted extraction of Artemisia sphaerocephala polysaccharides with conventional method: Molecule structure and antioxidant activities evaluation. *International Journal of*

Biological Macromolecules, *45*(5), 483–492. https://doi.org/10.1016/j.ijbiomac.2009.09.004

Wang, T., Liang, H., & Yuan, Q. (2011). Optimization of ultrasonic-stimulated solvent extraction of sinigrin from Indian mustard seed (*Brassica juncea* L.) using response surface methodology. *Phytochemical Analysis*, *22*(3), 205–213. https://doi.org/10.1002/pca.1266

Xue, Y., Wu, C. Y., Branford-White, C. J., Ning, X., Nie, H. L., & Zhu, L. M. (2010). Chemical modification of stem bromelain with anhydride groups to enhance its stability and catalytic activity. *Journal of Molecular Catalysis B: Enzymatic*, *63*(3–4), 188–193. https://doi.org/10.1016/j.molcatb.2010.01.018

Yan, M. M., Liu, W., Fu, Y. J., Zu, Y. G., Chen, C. Y., & Luo, M. (2010). Optimisation of the microwave-assisted extraction process for four main astragalosides in Radix Astragali. *Food Chemistry*, *119*(4), 1663–1670. https://doi.org/10.1016/j.foodchem.2009.09.021

Yanık, D. K. (2017). Alternative to traditional olive pomace oil extraction systems: Microwave-assisted solvent extraction of oil from wet olive pomace. *LWT—Food Science and Technology*, *77*, 45–51. https://doi.org/10.1016/j.lwt.2016.11.020

Yu, J., & Mikiashvili, N. (2020). Effectiveness of different proteases in reducing allergen content and IgE-binding of raw peanuts. *Food Chemistry*, *307*, 125565.

Zeković, Z., Pintać, D., Majkić, T., Vidović, S., Mimica-Dukić, N., Teslić, N., … Pavlić, B. (2017). Utilization of sage by-products as raw material for antioxidants recovery—Ultrasound versus microwave-assisted extraction. *Industrial Crops and Products*, *99*, 49–59. https://doi.org/10.1016/j.indcrop.2017.01.028

Zhang, B., Yang, R., & Liu, C. Z. (2008). Microwave-assisted extraction of chlorogenic acid from flower buds of *Lonicera japonica* Thunb. *Separation and Purification Technology*, *62*(2), 480–483. https://doi.org/10.1016/j.seppur.2008.02.013

Zinoviadou, K. G., Galanakis, C. M., Brnčić, M., Grimi, N., Boussetta, N., Mota, M. J., … Barba, F. J. (2015). Fruit juice sonication: Implications on food safety and physicochemical and nutritional properties. *Food Research International*, *77*, 743–752.

SUPPORTING INFORMATION

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