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Research Article

MICROWAVE METHODS FOR THE EXTRACTION OF BIOACTIVE COMPONENTS AND ENZYMES FROM PINEAPPLE WASTE AND ITS APPLICATION IN MEAT TENDERIZATION

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Abstract- The main purpose of this study is to extract the bioactive compounds from the pineapple waste using Microwave assisted extraction(MAE), as there is large amount of left over after processing which has potential uses. Bromelain a proteolytic enzyme found in the pineapple peel, stem, crown and core possess a lot of bioactive compounds like phenolics, antioxidants and enzymes which is useful in the food industry as well in pharmaceuticals. In this study MAE technique using water as solvent was employed to extract the bio actives and the proteolytic enzyme bromelain from the pineapple waste (peel and core) . Response surface methodology with Box Behnken design using the three independent variables time (min), power (watt) and solid to solvent ratio(g/ml) to determine the effect on the amount of Total protein, phenolic content and antioxidants (FRAP). Further using the optimized condition, the protein sample was purified using acetone to obtain Bromelain enzyme. This enzyme was freeze-dried, and applied to meat chunks as a tenderizer, and physiochemical parameters were determined and comparing it with the commercial bromelain from pineapple stem and results were analyzed.

Keywords- Microwave-Assisted Extraction, Pineapple waste, Response surface methodology Bromelain, Meat Tenderization.

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Introduction

Pineapple peel and core is the byproduct obtained by processing industries for the production of RTS, canned and minimally processed fruit [9]. It is found that 75 % of the whole fruit goes waste during processing. The by-products of tropical and subtropical fruit processing are usually more than that of the temperate fruits [34]. The common processing wastes from pineapple are peeled skin, core, and crown. These wastes have been an interesting source of an enzyme called bromelain, phenolic antioxidants and some by-products like organic acids, fibre, vinegar and biogas. Antioxidants are compounds capable of scavenging free radicals delaying, retarding or preventing auto-oxidation. The growing interest and awareness of the consumers natural foods and health have fostered more efficient and cleaner extraction processes to isolate natural antioxidants, phenolics and enzymes.

Besides all of these, the poor handling of fruits and exposure to various environmental conditions during transportation may lead to waste which accounts for 55% of product waste. Reports show that 40-80% of such wastes have high biological oxygen demand (BOD) and chemical oxygen demand (COD) values [32].

Bromelain, a proteolytic enzyme, present at the different parts of pineapple constitutes thiol-endopeptidases, phosphatases, glycosidase, peroxidases, glycoproteins, carbohydrates, cellulases, protease inhibitors, and organically bound calcium which are not characterised completely. The Stem and fruit bromelain have a molecular weight of 33 kDa with isoelectric point of 9.5 and 28 kDa with isoelectric point of 4.6 respectively [26].The extract of bromelain exhibits its activity in the pH range of 4.5–9.8[1]. It has lot of potential applications in baking industry [19], meat tenderisation [17], Fish protein hydrolysate [11], anti-browning agent [21]. Microwave assisted extraction is a trending novel green technological approach for isolating the bioactive compounds using water as a solvent. Electromagnetic radiation is directly applied to the organic solvents for the extracts which can absorb electromagnetic energy which is transformed into heat [12] in a technique. The MAE leads in quick processing, high yield and quality of product, extraction capability, lower consumption of energy, low amount of solvent, and less capital input compared to conventional extraction methods [10].

Meat tenderization is one of the factor which improving the quality of the meat [20], furthermore tenderness is the major factor which has affect on consumer's perception of meat taste and it adds better price to meat. Many researchers have been identified that protein degradation and protein oxidation can be used for meat tenderization [20], for the fact that united state federal agencies recognized papain, ficin, bromelain, Aspergillus oryzae, protease and Bacillus subtilis protease which are exogenous enzymes used for meat tenderization [5].

The objective of the present study is to investigate the extraction of bioactive compounds through MAE Technique using water as solvent and to determine the yield of protein, total phenolic content, antioxidant power using the three independent variables time (min), power (watt) and solid to solvent ratio (g/ml) by the response surface methodology to obtain the optimized condition and to characterize the bioactives. Further purification of protein was done and the enzyme bromelain was characterized, which was applied as a meat tenderizer and the physico chemical properties of the meat were analysed by comparing it with commercial bromelain from pineapple stem.

Materials and Methods

Materials:

Raw materials: Pineapple peel, core and meat chunks

Equipment's and glassware's:
Hot air Oven (Memmert) ,Mixer grinder, Microwave oven (Samsung,1.6 cu.ft, 1000W), Whatman filter paper no.1, Centrifuge tubes, Funnels, Centrifuge (Hsiangtai), UV spectrophotometer (UV-UNICAM,ALVA,U.K) ,Water bath ,pH-meter (Consort C 3010), Vortexmixer, Plastic cuvette, Micropipettes, Texture analyzer (TA-XT plus) ,Magnetic stirrer ,Spatula , Thermos-gravimetric analyzer, Petri dish , Pipettes ,Test tubes ,Beakers Volumetric flask ,Weighing balance (Mettler Toledo), Freeze dryer (Scanvac Coolsafe 55-4, Labogene, Lynge, Denmark).

Chemicals:
All chemicals used were of analytical grade.
Bradford dye reagent (PRD.0.ZQ5.10000050486) ,Sodium acetate, Glacial acetic acid(ARK2183), EDTA, HCl, Folin -ciocalteau reagent (Code No: -03870), Ferric Chloride, Gallic acid, TPTZ (2, 4, 6-tripyridyl-s-triazine), Potassium phosphate buffer, Glycerol, Ferric chloride, Methanol (CAS No: 67-56-1), Sodium carbonate (CAT No:463), Acetone, Bromelain from pineapple stem (CAS No: 37189-34-7), L-Tyrosine (CAS No:60-18-4), Casein (CAS No: 9000-71-9), Sodium carbonate solution, Sodium acetate buffer, Calcium acetate buffer, L-cysteine (PUB chem 24901592), Trichloroacetic acid (PubChem CID 6421) Sodium dodecyl sulfate (PubChem CID 3423265), Hydrochloric acid (PubChem CID 313) Sodium hydroxide (PubChem CID 14798).

Methodology:
Sample preparation: Pineapple waste such as peel and core was brought from the "Talad Thai market", Thailand. All the waste materials were cleaned using distilled water and oven dried at 40°C for 24 hrs to remove the moisture Further it was put in to the lab scale, mechanical grinder and grinded well. The obtained powder was stores at 4°C for further extraction and analysis.

Microwave assisted extraction: The experimental set up for microwave-assisted extraction (Samsung, 1.6 cu.ft,1000W) contained the heating unit, a flask for extraction (1L), condenser and a vacuum machine. In this experiment 5 g of powdered sample of (peel&core) was taken using distilled water as the extraction solvent. The flask with the sample was kept inside the microwave chamber and power was supplied at (100, 180 and 300 W) for the time (5, 10 and 15 minutes) and the solid to solvent ratio (1:10, 1:20,1:30 g/ml) . Design Expert Software (trial version 10.0.1, stat-Ease Inc, Minneapolx, MN, USA) was used to optimize the extraction conditions of pineapple peel and core. The response surface methodology (RSM) using box-benkhen design gave 15 experimental runs. Accordingly it evaluated the interactive effects of Time(X_1), Power(X_2), solid to solvent concentration(X_3) to the extracted amount of protein, TPC and FRAP of the pineapple core and peel. The experimental design in to randomized manner and the data was analyzed according to the quadratic polynomial regression.

$$Y = \beta_0 + \Sigma \beta_i X_i + \Sigma \beta_{ii} X_i^2 + \Sigma \beta_{ij} X_i X_j$$

In this equation, Y-Response variable; β0-Constant; βi- Linear coefficient; β_{ii}-Quadratic coefficient; β_{ij}-interactive coefficient; X_i, X_j-independent variables.

Table-1 *Independent variables according to the coded and actual values for MAE treatment for peel and core*

Symbol	Independent variables	Units	Coded level		
			-1	0	1
X_1	Time	min	5	10	15
X_2	power	watt	100	180	300
X_3	Solid: solvent ratio	g:mL	10	20	30

Acetone precipitation for the purification of protein:
In this present study acetone precipitation method [24] was done to obtain pure samples of protein. The volumes of Acetone and the pineapple crude extract were taken as 4:1 ratio. The required volume of acetone was cooled to -20 C◦ before being used, then the pineapple crude extract was put in the centrifuge bottle and acetone was mixed and shaken vigorously. The sample was then kept at -20 °C for 60 min further it was centrifuged for 10 min at 13,000g then after the supernatant was discarded and centrifuge bottle was placed in inverted direction for 15 min to remove excess acetone from the protein pellet and then it was re-dissolved in 0.01M sodium phosphate buffer(PH.7.0) and vortex thoroughly.

Freeze drying of Bromelain:
Freeze drying is the most commonly used technique for preparing the bromelain powder [8]. In our study after the desaltation process using acetone, the pure sample was put in a petri dish and kept in a freezer (-14 °C) for 12 hrs. Further, it was subjected to freeze drying (Scanvac Coolsafe 55-4, Labogene, Lynge, Denmark) for 24 hrs at -55°C. Studies states that after freeze-drying there is probably an increase in the bromelain activity [28], this can be because drying at lower temperature reduces the risk of protein denaturation.

Preparation of meat samples:
The chickens were brought from the "Macro supermarket" in Thailand. Chicken breast was cut in to uniform slices of 1×1×1 cm and weighed. Then after the bromelain obtained from MAE treatment which was freeze dried and the commercial bromelain (CASNo: 37189-34-7) was prepared at different concentrations (0%, 3%, 5% and 7%) (W/W) was applied to meat .The treated chunks of meat were put in to the plastic box and kept for 60 min in room temperature[15]. All the other physio-chemical characteristics were determined after this process.

Analysis:
Quantitative Analysis:
The total protein content of the sample was determined using the Bradford method. In this Braford standard was taken as reference in the sample determination, by measuring the absorbance at 595 nm using UV Spectrophotometer (UV-UNICAM, ALVA, U.K) [2] the protein content was determined. The phenolic content of the sample was measured by taking gallic acid as a standard using Folin-Ciocalteau method[18], the absorbance of the sample was measured at 765 nm UV Spectrophotometer (UV-UNICAM, ALVA, U.K). The Ferric reducing antioxidant power (FRAP) content of the sample was obtained by taking Ferrous sulphate as standard. FRAP reagent was freshly prepared and mixed with sample and vortexed thoroughly [37]. The mixture was measured at 593 in the UV Spectrophotometer and FRAP content was determined.

Determination of proteolytic activity:
The proteolytic activity of the bromelain extract was determined [25] using method. This assay is based on colorimetric method [35]. In this process, L- tyrosine is used as a standard in which the bromelain breaks up the smaller peptide bonds present and helps in releasing the amino acids, which are free after hydrolyzing with protein. Bromelain activity was determined using the casein as substrate in the presence of cysteine and EDTA. In this method, L-Tyrosine is released from casein after hydrolysis with bromelain enzyme, further the unhydrolyzed substrate was precipitated using TCA. The precipitate obtained was filtered using a syringe filter and the filtrate was used in color development. The absorbance was measure at 275 nm.
The enzyme activity was calculated using:
Activity(CDU/ml)= $Et - \frac{Eb}{Es} * concentration\ of\ standard\ Ltyrosine * \frac{Vr}{Tr} * Df$
Where
E_t - absorbance of enzyme sample,
E_b - absorbance of enzyme blank and
E_s - absorbance standard L- tyrosine
D_f - dilution factor
Vr - reaction volume
tr - reaction time.

Physico chemical analysis of meat treated with Bromelain:
Moisture content: Moisture content of the meat sample was determined by [27]
Moisture content = [(Empty weight + sample weight)-dry weight] / sample weight ×100

Cooking yield

The treated rectangular shaped chicken chunks were weighed accurately and steamed for 5 min. Then the cooked chunks were re-weighed and calculate for the cooking yield according to the following equation [38]

Cooking yield (%) =[(weight of cooked chunks/weight of raw chunks) ×100]

pH:

To determine the pH of the sample, the treated chicken chunks samples were homogenized using 10 ml of chilled distilled water [15]. Then the pH was measured using the digital pH meter (Consort C 3010).

Texture analysis:

The texture Analyzer (TA-XT plus) was used to check the textural properties of meat. In accordance to the method described [15], Rectangular shaped treated chicken chunks was pressed down at a constant speed of 2mms-1.Then maximum Shear force (N) of meat which were treated at different concentrations were recorded accordingly.

Statistical Analysis:

Statistical analysis was done by using SPSS statistical software package (SPSS Version 16, Chicago, IL, USA). All experiments were means the obtained from the triplicate data. The significant differences between the means ($p<0.05$) were evaluated by ANOVA and Duncan test.

Results and Discussion

Model fitting of Microwave assisted extraction of bioactive compounds from peel:

Response surface methodology was used as a technique to determine the optimum conditions for the extraction of bioactive compounds. The effects of time, power and solid-to-solvent ratio on the protein, phenolics and antioxidants were observed using the Box-Behnken design. The results which were obtained from the 15 runs are given in [Table-2]. The results obtained ranged from Protein (3997.67-10442.28 µg/g), phenolic content (5140.67-13016.4 µgof GAE/g), FRAP content (58.60-120.57µmol/g). Pineapple has the active component present in it, is a protein digesting enzyme called bromelain, which is a rich source of cellulose, hemicellulose and carbohydrates. The protein content differs from the variety of pineapple, type of extractant, extraction technique used.By means of membrane processing the protein content was found to 1.37 mg/ml [29], when characterizing the protein from peel of different varieties of pineapples Nang Lae (NL) peel when extracting using distilled water has protein content of 0.337 (mg/ml) and Phu Lae (PL) using the same extraction and solvent gave a protein content 0.215 (mg/ml) [16]. The major polyphenols present in the pineapple peel are the catechin, epicatechin, ferulic acid and gallic acid, phenolic content of the peels was found to be 31.98 mg gallic acid equivalents (GAE)/g extracts in his study [22]. Various studies reveal that the phenolic and antioxidant value obtained from the microwave conditions are higher because microwaves has the ability to disrupt hydrogen bond networks The microwave-induced dipole rotation of molecules, and the migration of ions that enhance the penetration of solvent in to matrix, disrupts the cell wall and releases the intracellular product, allowing for the extraction of different components [31] The total phenolic content in the potato peel using methanol as solvent in the MAE was found to be 3.94 ± 0.21 mg /g dw [36].

From the responses in this present study, it can be predicted that it has an impact on the extract conditions. Therefore, to check the adequacy of the model among the model terms Analysis of variance (ANOVA) was done. The coefficient of determination (R^2) and the adjusted-R^2 were calculated to evaluate the adequacy and fitness of the model. The lack of fit was also performed to determine which model can explain the experimental data [40]. Regression analysis was done to fit the response model for all responses and also the linear and quadratic terms of the independent variables [14]. Therefore, the regression equation represents an empirical relation between the responses and the independent variable are given below

$$Y_1=6961.68 + 459.924\ X_1 + 894.659\ X_2 + 2289.61\ X_3 + 168.002\ X_1X_2 + 89.4658\ X_1X_3 - 544.788\ X_2X_3 - 109.984\ X_1^2 - 697.094\ X_2^2 - 104.122\ X_3^2$$

$$Y_2=7071.42 + 1552.73\ X_1 + 1730.77X_2 + 434.095\ X_3 + 1424.73\ X_1X_2 + 180.18\ X_1X_3 - 663.673\ X_2X_3 - 114.922\ X_1^2 - 660.276\ X_2^2 - 52.5523\ X_3^2$$

$$Y_3=92.8878 + 1.74503\ X_1 + 7.45014\ X_2 + 21.2046\ X_3 + 1.18882\ X_1X_2 - 1.81837\ X_1X_3 - 3.4055\ X_2X_3 - 1.3456\ X_1^2 - 3.08581\ X_2^2 - 2.81068\ X_3^2$$

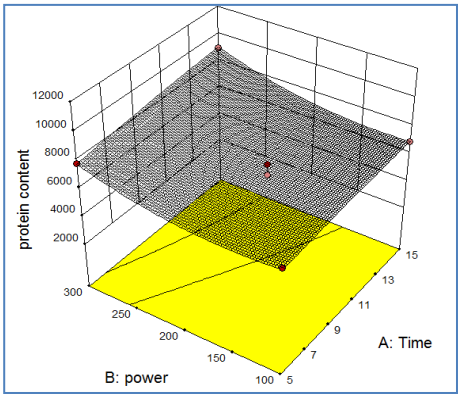
Here Y_1, Y_2, Y_3 represent the Protein content, phenolic content and the Frap content of peel and X_1, X_2 and X_3 are the actual values of the independent variables.

From the ANOVA response, the adequacy of the model was predicted. It showed that Time (X_1), Power (X_2), solid -to-solvent ratio(X_3) had a significant effect ($p<0.05$) on the protein content, TPC and FRAP. In determination of FRAP content the time was not significant. However, the model was found to be significant for all the three responses and the lack of fit was found insignificant with p-values of 0.96, 0.58, 0.89 respectively. This imply that the model fitted is good for predicting and determining the values for protein content, total phenolic content and antioxidant content within the design space. Regression values (R^2) was used to determine the fitness of the model and it was found that the R^2 for protein content, TPC and FRAP were 0.9917, 0.9611 and 0.8926 respectively which resulted in a good fit of the empirical model with the experimental data [14].

The optimum extraction condition was determined using the desirability function which gave various solutions from the design expert. They were time 15 min, power 300 Watt and solid to solvent ratio of 1:29 g: mL which the maximum protein was 10961.34(µg/g), TPC 12456.50 (µg of GAE/g) and FRAP 120.62 (µmol/g) with the desirability of 0.976. To check the validity of the predicted value experiment was done using the optimized condition obtained and it was found that protein, TPC and FRAP were 8827.30(µg/g), 12441.11 (µg of GAE/g) and 120.64 (µmol/g) respectively, therefore these experimental values were nearly similar.

In order, to determine the interactive effects between the independent variables and responses, a response surface plot was generated by keeping one variable as constant and other two were varied to show the effects in [Fig-1, 2 and 3].

a.)



b.)

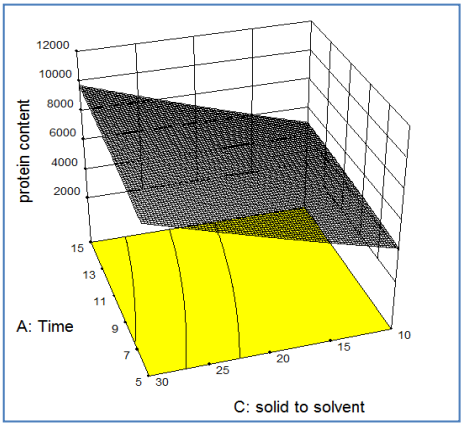


Table-2 *MAE of peel: Box behnken experimental design and predicted responses for protein content, phenolic content and antioxidant content (FRAP)*

Independent Variables				Response variables					
Run order	Time (X_1) min	Power (X2) Watt	Solid: Solvent (X3) g:mL	Protein (µg/g)		TPC (µg of GAE/g)		FRAP (µmol/g)	
				Experiment	Predicted	Experiment	Predicted	Experiment	Predicted
1	10	180	20	7077.75	6810.63	7453.91	6751.67	100.49	91.52
2	15	300	20	8974.12	9071.37	13016.4	12554.84	105.07	107.70
3	10	180	20	6282.49	6810.63	6040.19	6751.67	89.94	91.52
4	5	180	30	8634.61	8687.54	6343.72	5932.91	109.97	112.25
5	10	300	10	6967.63	6912.73	9506.58	9639.49	83.62	82.81
6	10	180	20	7071.63	6810.63	6760.91	6751.67	84.12	91.52
7	15	180	30	9790.78	9719.12	8575.19	8828.84	112.21	111.63
8	10	300	30	10442.28	10402.37	8921.69	9180.33	120.57	118.41
9	15	100	20	6918.70	6946.05	6446.75	6243.85	90.19	90.43
10	15	180	10	4795.98	4743.06	6924.00	7334.82	73.77	71.50
11	10	100	30	9643.97	9702.63	7147.60	7046.14	109.85	110.32
12	5	300	20	7817.94	7815.52	6529.91	6599.93	101.49	101.84
13	5	100	20	6484.37	6362.20	5393.39	5987.84	92.51	89.31
14	5	180	10	3997.67	4069.34	5413.25	5159.61	64.25	64.84
15	10	100	10	3997.67	4033.84	5140.67	4850.60	58.60	61.10

c.)

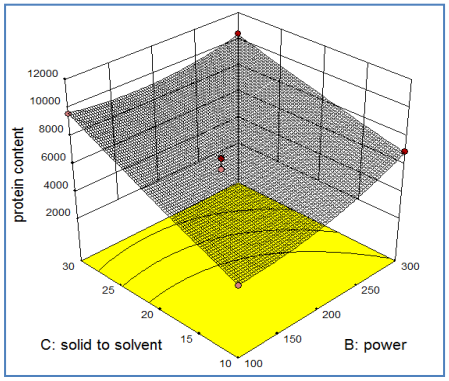


Fig-1 Response surface plots showing the interactive effects of time (min), power (watt), solid to solvent ratio (g:mL) on protein content from pineapple peel a.) Time& power b.) Time & solid to solvent c.) power & solid to solvent

c.)

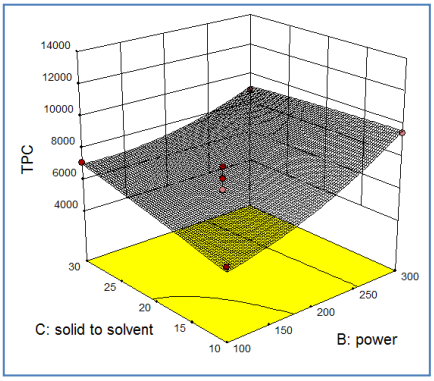
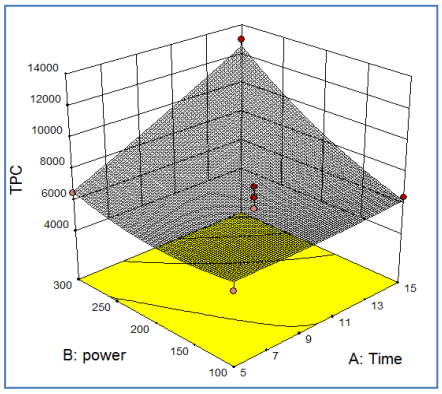
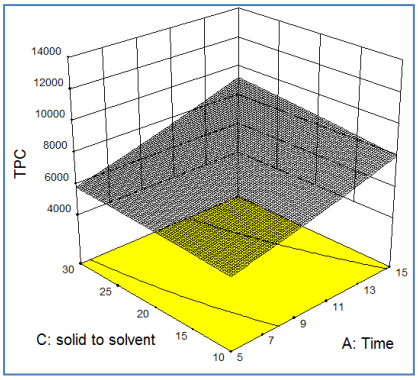


Fig-2 Response surface plots showing the interactive effects of time (min), power (watt), solid to solvent ratio (g:mL) on TPC of pineapple peel a.) Time& power b.) Time & solid to solvent c.) power & solid to solvent

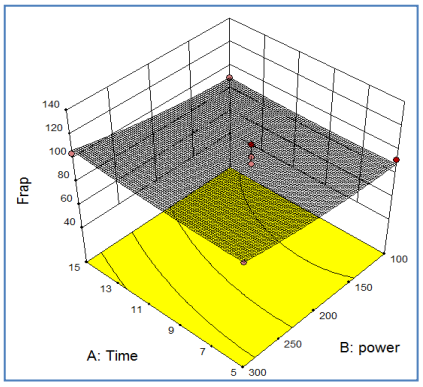
a.)



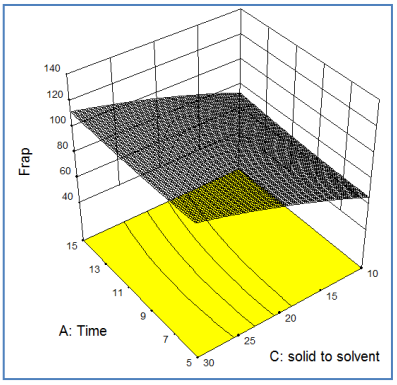
b.)



a.)



b.)



c.)

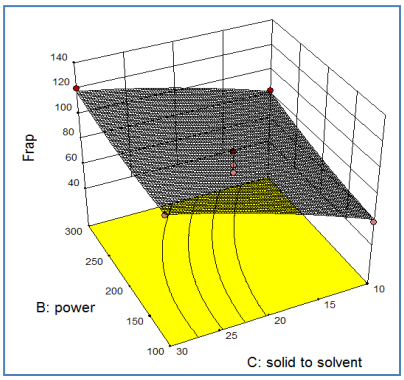


Fig-3 Response surface plots showing the interactive effects of time (min), power (watt), solid to solvent ratio (g:mL) on FRAP of pineapple peel a.) Time& power b.) Time & solid to solvent c.) power & solid to solvent.

Model fitting of Microwave assisted extraction of bioactive compounds from core:

From the extraction conditions the results from [Table-3], ranged from protein (2924.08 - 8047.34 µg/g), total phenolic content (2405.63 -5330.09 µg of GAE/g) and frap content (53.82 - 154.48 µmol/g). Correlating the results obtained in this present study to the previous studies of the past states that the protein content of core depends on the extraction buffer pH, temperature and strength of extraction buffer[5], the protein content was 12.6 mg/ml, the phenolic content on the core 10mg/g GAE [41] using ethanol as solvent. Whereas FRAP value of the pineapple core was found to be 2.01 mmol/100 [13].

Various studies on the microwave technique to extract the polyphenols proved to efficient, good selectivity and improved recovery yields and purity of the crude extracts [7]. The extraction yield of total polyphenols using microwave technique (1.75%) was higher than other extraction methods including heat-refluxing extraction (1.53%), ultrasonic-assisted extraction (1.56%) and enzyme-assisted extraction (1.62%) [42]

Table-3 *MAE of core: Box behnken experimental design and predicted responses for protein content, phenolic content and antioxidant content (FRAP)*

Independent Variables				Response Variables					
Run	Time (X_1) min	Power (X2) Watt	Solid:Solvent (X3) g:mL	Protein(µg/g)		TPC(µg of GAE/g)		FRAP(µmol/g)	
				Experiment	predicted	Experiment	Predicted	Experiment	Predicted
1	10	180	20	4997.85	4914.26	2658.35	3157.31	65.66	78.00
2	15	300	20	5046.79	5178.28	4737.35	4725.76	92.10	104.11
3	10	180	20	4783.75	4914.26	3194.27	3157.31	78.05	78.00
4	5	180	30	7028.81	7026.11	2740.12	2719.74	97.75	105.53
5	10	300	10	2933.26	2799.62	5330.09	5325.39	81.17	73.79
6	10	180	20	4961.15	4914.26	3619.31	3157.31	90.27	78.00
7	15	180	30	8047.34	7775.22	3571.72	3280.03	144.13	138.01
8	10	300	30	7405.02	7491.26	3530.14	3775.10	154.48	147.39
9	15	100	20	4887.74	5025.70	3009.47	3292.38	82.95	82.86
10	15	180	10	2746.68	2749.38	3227.99	3248.38	63.21	57.45
11	10	100	30	7065.51	7254.12	3876.64	3943.77	111.09	118.55
12	5	300	20	4948.92	4864.85	4053.59	3824.95	83.20	85.69
13	5	100	20	4948.92	4763.56	3009.47	2966.80	79.55	65.17
14	5	180	10	2661.03	2933.17	2405.63	2697.33	49.83	55.96
15	10	100	10	2924.08	2782.89	3172.55	2865.19	53.82	60.85

From the ANOVA response it was observed that solid to solvent ratio (X_3) had a significant effect on the protein and the FRAP content. Power (X_2) had a significant impact on the phenolic content. Time (X_1) had no significance in all three cases. Henceforth the model was found to be significant for the responses such as protein and FRAP whereas in case of TPC the model was found insignificant. Lack of fit test was found to be insignificant with p values 0.11, 0.64, 0.41 respectively. This shows the fitted model is good in case of determining the protein content and FRAPS content within the design. Therefore, the coefficient of determination (R^2) were 0.9922 for protein content, 0.8918 for total phenolic content and 0.9172 for FRAP content states a good fit for the empirical data.

The multiple regression equation which represents the empirical relationship is given below

$Y_4=4927.38 + 143.892\ X_1 + 63.4673\ X_2 + 2290.72\ X_3 + 12.8196\ X_1X_2 + 233.223\ X_1X_3 + 55.1007\ X_2X_3\ B + 41.4195\ X_1^2 -10.7\ X_2^2- 165.295\ X_3^2$

$Y_5=3244.66 + 306.596\ X_1 + 572.881\ X_2-117.928\ X_3+ 143.809\ X_1X_2 + 2.31\ X_1X_3 - 657.218\ X_2X_3 -222.915\ X_1^2- 680.729\ X_2^2 - 51.975\ X_3^2$

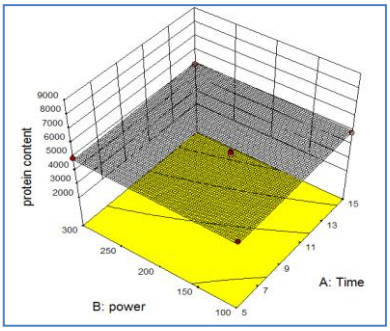
$Y_6=79.8028 + 9.02687\ X_1 + 10.4426\ X_2 + 32.8267\ X_3+ 0.181306\ X_1X_2+ 8.24987\ X_1X_3+ 3.97479\ X_2X_3 -2.47651\ X_1^2 -7.12919\ X_2^2 - 13.2132\ X_3^2$

Here Y_4, Y_5, Y_6 represent the Protein content, phenolic content and the Frap content of core and X_1, X_2 and X_3 are the actual values of the independent variables.

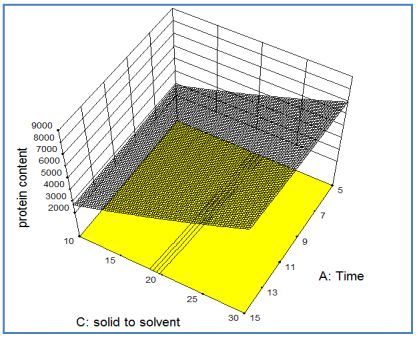
In this study using the desirability function, the most desirable solution was 0.911. The optimized condition obtained were time of 15 min, Power of 300 Watt and solid to solvent ratio 1:30 g:mL at which maximum protein was 7922.61 µg/g , 4004.89 µg of GAE/g and 162.37 µmol/g. Experimentally the protein content, TPC and FRAP were 7946.41 µg/g, 7078.30 µg of GAE/g and 117.95 µmol/g respectively.

Therefore, to determine the interactive effects between the independent variables and responses a response surface plot was generated by keeping one variable as constant and other two were varied to show the effects in [Fig-4, 5 and 6].

a.)



b.)



c.)

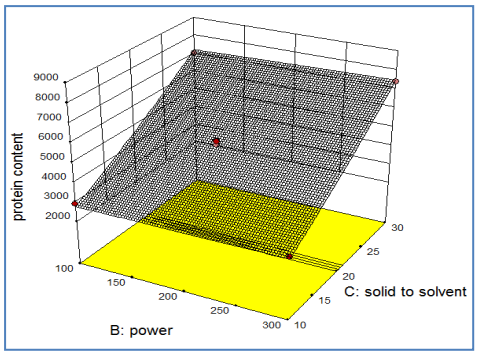
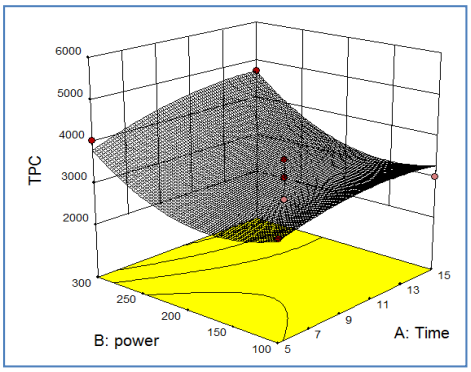
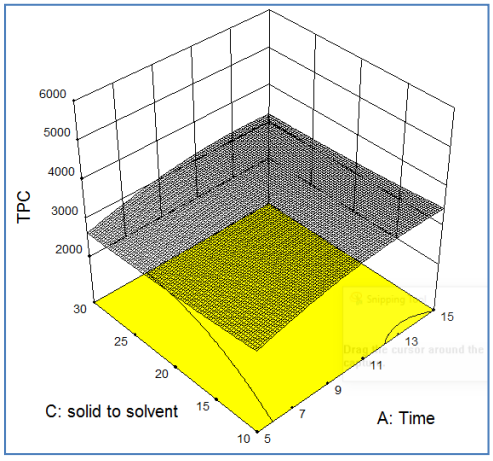


Fig-4 Response surface plots showing the interactive effects of time (min), power (watt), solid to solvent ratio (g:mL) on protein content from pineapple core a.) Time& power b.) Time & solid to solvent c.) power & solid to solvent

a.)



b.)



c.)

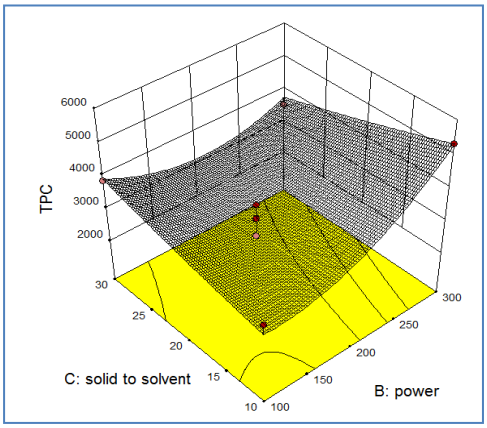
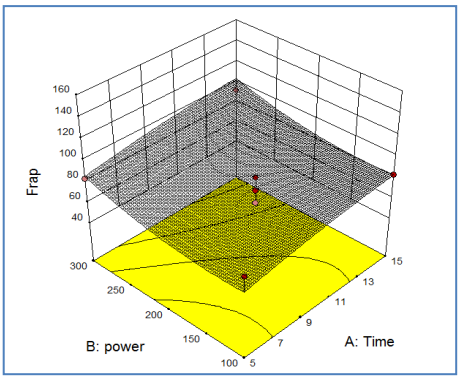
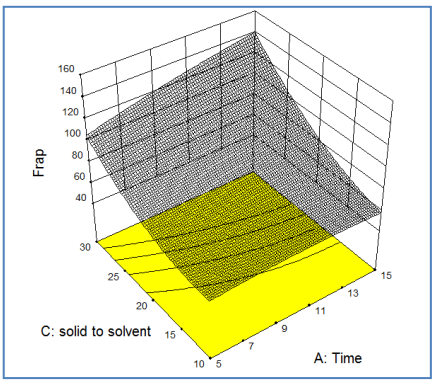


Fig-5 Response surface plots showing the interactive effects of time (min), power (watt), solid to solvent ratio (g:mL) on TPC of pineapple core a.) Time& power b.) Time & solid to solvent c.) power & solid to solvent

a.)



b.)



c.)

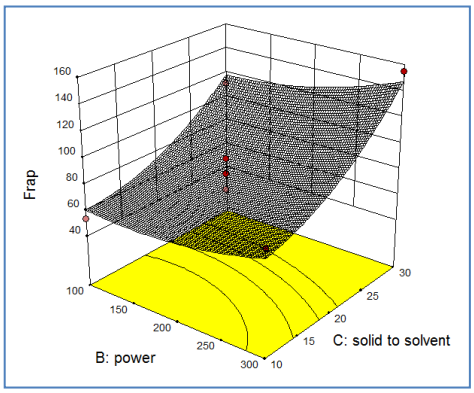


Fig-6 Response surface plots showing the interactive effects of time (min), power (watt),solid to solvent ratio (%) on FRAP of pineapple a.) Time& power b.) Time & solid to solvent c.) power & solid to solvent

Bromelain activity:

In the present study, bromelain activity was determined for the optimized extraction condition obtained from the MAE as in [Table-4]. Studies revealed that extraction of bromelain depends on the extraction solvent, temperature and pH used [5]. Bromelain activity was mainly dependent on the variety of pineapple and extractant solve used this is probably due to the ability and quantity of active compounds contained in the sample [16], most of the proteases present in the pineapple belong to the cysteine proteases and contain cysteine residue in their active sites, so when there is a extractant with the particular activator gave a high enzyme recovery [6]. Using Distilled water with cysteine the Nang Lae(NL) variety of pineapple gave a total enzyme activity of 321 units while the Phu Lae (PL) variety gave 478 units [16], this clearly states that type of variety and extraction solvent plays a major role in determining the bromelain activity. Similarly using distilled water (DI) the bromelain activity of NL peel and PL peel were 327.71 units and 443.66 units respectively this correlated with the present study using MAE technique and water as solvent gave a higher bromelain activity. This can be because of the novel technique used in our study.

Desalting of proteins to obtain the pure protein was performed using Acetone (99.5%) which gave a purification fold of 1.34 and 1.11 for peel and core

respectively. In studies [5] stated that acetone precipitation gave a higher enzyme recovery with purification fold ranged from (2-5) depending on the concentration of acetone used than the ammonium sulfate precipitation. Similar results were observed in this study using MAE technique, water was used as solvent it was found that peel had a higher protein content and bromelain activity with purification fold of 1.34.

Table-4 *Bromelain activity for the optimised condition of the pineapple peel and core*

	Before purification			After purification			
Pineapple Waste	Total protein (mg/g)	Total enzyme (units/ml)	Specific Activity (unit /mg protein)	Total protein (mg/g)	Total enzyme (units/ml)	Specific Activity (unit /mg protein)	Purification fold
Peel	8.8 ± 0.34	400.66± 0.5	45.52	10.1 ± 0.05	620.35 ±0.3	61.38	1.34
Core	7.9 ± 0.22	387.55 ±1.8	49.0	9.2 ± 0.1	500.66 ± 0.01	54.41	1.11

Values are the means ± standard deviation (SD) obtained from the triplicate data
Specific Activity: total enzyme activity/ total protein.
Fold Purification: Specific activity after purification/initial specific activity before purification

Meat tenderization:

The sample protein obtained from the optimized condition was purified, comparatively the protein content and bromelain activity of peel was found higher than the core. So the protein from peel was used for meat tenderization in this study. Henceforth the pure sample of protein obtained after purification with acetone was freeze dried to obtain bromelain in the powder form. Further to study the effects of freeze dried bromelain, commercial Bromelain procured from Sigma Aldrich (CAS 37189-34-7) was taken to compare the physio chemical properties Both the type of bromelain were applied to the meat samples at these concentrations such as (0,3,5,7%w/w) and were determined and compared as shown in [Table-5].

Cooking yield:

From the results in [Table-5], it was observed that cooking yield has decreased as the concentration of the bromelain increased in the two types of bromelain used in this study. This can be due to the addition of bromelain extract at different levels could possibly be the tenderizing effects of proteolytic enzymes from the pineapple peel and also decrease in yield can be due to degradation of sarcoplasmic and myofibrillar protein in the meat [15]. ANOVA stated that there was no significant difference ($p>0.05$) was found on the different concentration of freeze dried bromelain, in case of commercial bromelain there was significant difference ($p<0.05$) was found at 5 % and 7 % bromelain concentration. However, there was no much difference on the cooking yield (%) from the bromelain obtained from MAE treatment and the commercial bromelain procured.

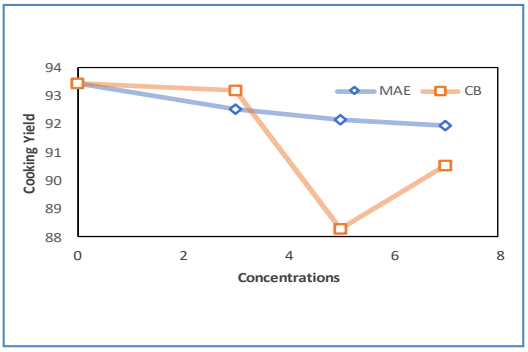


Fig-7 The effect of different concentration (%) of MAE Bromelain and Commercial Bromelain (CB) on the cooking yield (%)

Table-5 *Physio chemical characteristic of treated meat chunks*

	Parameters	Concentration %			
		0% (control sample)	3%	5%	7%
Freeze dried Bromelain from MAE	Cooking Yield (%)	93.46±0.393^{a}	92.51±0.246^{a}	92.15±1.107^{a}	91.93±4.132^{b}
	Moisture (%)	73.15±0.328^{a}	71.53±0.365^{ab}	70.83±1.557^{ab}	69.42±3.312^{b}
	pH	6.66±0.0378^{a}	6.643±0.143^{a}	6.52±0.115^{a}	6.55±0.493^{a}
	Force (N)	22.42±1.017^{a}	22.33±4.85^{a}	19.593±7.17^{a}	19.45±4.969^{a}
Commercial Bromelain	Cooking yield (%)	93.46±0.393^{c}	92.87±0.273^{c}	88.26±0.701^{a}	90.51±0.459^{b}
	Moisture (%)	73.15±0.328^{d}	72.79±0.111^{c}	72.43±0.086^{b}	71.13±0.112^{a}
	pH	6.66±0.0378^{c}	6.60±0.000^{b}	6.59±0.115^{b}	6.53±0.057^{a}
	Force (N)	22.42±1.017^{d}	15.18±0.010^{c}	12.25±0.050^{b}	10.66±0.020^{a}

Moisture Content

The moisture content of the bromelain treated samples significantly decreased compared with the control sample in both the type of bromelain used for the treatment from [Table-5], this clearly proved that enzyme treatment improved hydrophilic properties [15], it can also be because of the hygroscopic nature of the bromelain powder, which can absorb the moisture from the samples wet surface. The low moisture content is related to product acceptability and the shelf life extension for the consumer and industry [15]. ANOVA depicted that there was no significant difference ($p >0.05$) on the concentration (%) of the bromelain from MAE, but there was significant difference between control samples (0%) and the (7%) concentration treated meat samples. In case of commercial bromelain there was significant different in all the concentration (3, 5 & 7 %). However, as the bromelain concentration increased there was a reduction in moisture content (%) for both the bromelain types by improving the meat quality

pH

The pH value of the meat is a very significant because it can influence other quality properties such as WHC, tenderness and juiciness [z39]. In the current study it was observed that there was there was a decrease in pH remarkably in the bromelain treated sample compared with the control sample in both the type of the bromelain used. In a normal living muscle, the pH is approximately 7.2 [23].

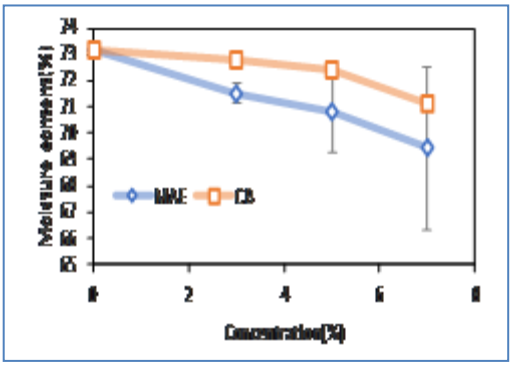


Fig-8 The effect of different concentration (%) of MAE Bromelain and Commercial Bromelain (CB)on the moisture content (%)

In MAE Bromelain the results from [Table-5], observed that pH decreased from (6.66 – 6.55) from the control to 7 %, same as in case of commercial bromelain pH decrease from (6.66 – 6.53). This decrease in pH could be because of the release of amino acids by the proteolytic activity of bromelain on meat proteins [4].

In case of MAE Bromelain, ANOVA showed that there was no significant difference found at different concentration($p > 0.05$) , but in case of commercial bromelain there was a significant difference ($p < 0.05$) in different concentration. At 7 % concentration of both the samples, the lowest at pH 6.55 was observed at MAE bromelain and 6.53 commercial bromelain. Studies reported that pH value below 5.8 increased the shelf life of vacuum packed meat, Because less pH inhibits the growth of the microbial species, which are responsible for the "greening" and development of off flavor in the packed meat [4].

The acidity of the meat denotes that the muscle fibres of the meat have been softened thus, lesser the pH, higher is the tenderization of the meat. [23]

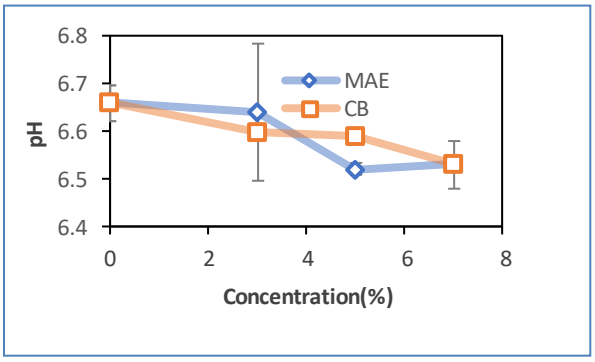


Fig-9 The effect of different concentration (%) of MAE Bromelain and Commercial Bromelain (CB) on the pH

Shear Force:

In the current study it was observed that force values significantly decreased in both the type of Bromelain(MAE & CB) treated when compared to the control without the Bromelain addition.

It was observed that the shear force values continuously decreased in all of the treated samples when the level of Bromelain increased. In case of MAE Bromelain it decreased from (22.42 – 19.45) in case of commercial bromelain it was (22.42 – 10.46) , ANOVA depicted that there was no significant difference in concentration when the sample was treated with MAE Bromelain , but in case of Commercial bromelain there was significant difference in the concentration with regard to the force. Similar studies [15] states that reduction of meat firmness results by the action of the proteolytic enzymes on myofibrillar proteins. Breakdown of myofibrillar protein occurred,small peptides, or proteins with low molecular weight (MW), were generated and resulted in reducing the firmness of the meat samples[15].

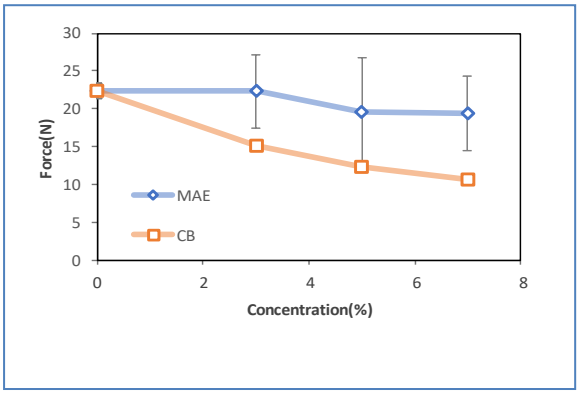


Fig-10 The effect of different concentration (%) of MAE Bromelain and Commercial Bromelain (CB) on the force

Conclusions

Microwave assisted extraction technique can be used effectively for the extraction of bromelain and other bioactive compounds from the pineapple peel and core. Using the optimized condition bromelain activity was determined. The extracted protein and bromelain activity was found to be higher in peel than the core, so for further purification peel was taken and purified with acetone also revealed that peel has higher bromelain activity and purification fold of 1.34, so the enzyme from peel was utilized for further study. The purified bromelain obtained from pineapple peel obtained from Acetone purification technique showed good tenderizing ability when applied to meat sample, comparing with commercial bromelain from pineapple stem with the same concentration as of MAE bromelain showed that there was not much difference on the physicochemical properties. Thus this study stated the effective utilization of waste from pineapple by obtaining the active components and extending its application to meat tenderization.

Author Contributions: All authors contributed equally

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Conflict of Interest: None declared

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