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BIOCHEMISTRY

Optimization of Ultrasonic Assisted Extraction of Bioactive components from different Parts of Pineapple Waste

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ABSTRACT

Waste accumulation is a serious problem in recent times as they cause serious effects to the environment. Pineapple waste is one among them and it includes the peel, core, stem and crown, which is obtained after processing and various bioactive compounds such as polyphenols, antioxidants and the protein digesting enzyme bromelain, are also present in pineapple waste. In the present study, the extraction of bioactive compounds from the pineapple peel and core was done using Ultrasound assisted extraction(UAE). The extraction conditions was optimised using the Response surface methodology by using the variables time(min), amplitude (%) and ethanol concentration. Protein samples obtained were purified using acetone precipitation and dialysis to determine the bromelain activity and protein pattern along with activity staining to confirm presence of bromelain enzyme.

Highlights

- High amount of polyphenols and enzymes were obtained from the peel and core using the ultrasound assisted extraction.

Keywords: Pineapple waste, Ultrasound assisted extraction, optimized condition, bromelain activity, purification

Pineapple (*Ananas comosus*) is the most valuable fruit which is ubiquitous and it is prime member of the family *Bromeliaceae* which is originated from South America. The production is 18.7 million tons in 2014 as per FAO. Its most preferred juice around the world next after apple and oranges. The growth of the plant is about the height of 75-150 cm and wideness of 90-120 cm which is short, stout stump with narrow, fibrous and spiny leaves[23]. Development of the plant was from cone-shaped from juicy and fleshy fruit with crown at the top part. Thailand is one of the gargantuan exporter of canned pineapple all over the world the year 2008 produced about 2.5 million tons reported in FAO and most of which is exported in the form of canned pineapples and as juices.

In pineapple processing sector, before peeling the fruit they cut off the stem, crown and core is being removed. Waste parts of pineapple before and after processing include core, stem, peel, crown which record commonly a 50% wastage of the whole pineapple weight. While the production of pineapple increasing day by day, the waste from the pineapple is also apparently increasing. The waste possess a lot of value and it is a known fact that it is enriched with cellulose, hemicellulose, carbohydrates, antioxidants, polyphenols and also the bromelain a protein digesting enzyme, is present in the pineapple waste[7]. Bromelain enzyme is greatly used in medical treatments because of its higher therapeutic value. It can be used as ananti-inflammatory agent which is used for the reduction of sports injury, trauma, arthritis, and swelling pains especially in athletic injuries and also in digestive problems and also for healing purpose for post-surgery[2]. The usage of bromelain helped in

reducing the arthritis, gout haemorrhoids, menses pain, auto immune disorders and ulcerative colitis,[1] of platelet clumping and blood clots in the blood stream, especially in the arteries[26].

Ultrasound assisted extraction is a simple, innovative, cost less and efficient substitute to the conventional extraction process. The increase in extraction yield using ultrasound is mainly put down due to the effect of acoustic capitations created in the solvent through which the ultrasound wave is passed through. The mechanical effect produced in the ultrasound helps in penetration of solvent into the sample by increasing the surface area and the contact between the solid and liquid phase, this will result in quick movement of the solute from solid phase to the solvent[18]. Ultrasound-assisted extraction is more remarkable because there is less chance chemical degradation in the targeted bioactive[19].

Disposal of waste is an extending issue, as there is microbial spoilage acting on and that causes serious environmental issues. Waste from the food industry possess a lot of values and the pattern of waste management and disposal in the food industry is an integrated approach in the scene of recycling and reuse in the waste recovery. Pineapple waste would be an excellent source of proteolytic enzymes, phenolic antioxidants. Convention extraction technique such as maceration is more of time consuming, and yield is less compared to other novel techniques[8].

The objectives of this present research were to investigate, the yield of protein, total phenolic content, antioxidant power using the variables time(min), amplitude(%) and ethanol concentration (%) through UAE treatment. Further, the study aimed to identify the optimum operating conditions for the processing of peel and core by using the response surface methodology and determine the yield through the characteristics of the bioactive compounds.

MATERIALS AND METHODS

Raw materials

Raw materials used in this experiment is the pineapple peel and core obtained from the "Prime Products Industry Co. Ltd" Chonburi Province, Thailand, which is the prime areas for the pineapple plantations. The factory produces various products such as canned pineapples which includes titbits, chunk, slices, pieces and also fruit cocktail and juices. Through these products, the company generates a waste of 13% percent which can be utilized efficiently.

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, 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).

Methods

Preparation of the sample

The collection of Pineapple waste (peel and core) was done from the "Prime Products Industry Co. Ltd" The samples was cleaned with distilled water and made into small pieces separately which was dried in the hot air oven. The dried samples were grinded in a lab scale mechanical grinder into a made into a piece of fine powder and kept at 4 °C for further process.

Ultrasonic assisted extraction

The bioactive components from the pineapple (peel and core) was obtained by using the ultrasound probe (UP 200S, 200W, HIELSCHER, TELTOW, Germany) using different concentration of ethanol (0, 20 and 40 %) as solvent. A fixed ratio of sample to solvent was used (1:10). The operation of ultrasound was at mode of 0.5 pulser. The process of extraction was performed 3 different time intervals (10, 20, and 30 min) There was also a condenser which helped in maintaining the solvent temperature below 15°C. In a beaker of 200 ml or 250 ml the sample was added and kept in the ultrasonic extractor. A little amount

of gap was there to make sure that probe doesn't touch the beaker, although the probe is dipped in the beaker.

In this process of UAE, RSM was used to optimize and reduce the trial to 15 conditions to extract proteins, polyphenols and antioxidants from the pineapple peel and core. The independent factors were time (A: 10 min, 20 min and 30 min) Amplitude (B: 60%, 80% and 100%), and ethanol concentration (0, 20 and 40%) and responses were total protein content, total phenolic content and antioxidant activity. The above-mentioned factors to be optimized were coded at three levels −1, 0 and 1. The three-factor Box-Behnken data design reduced the number of experimental trials to 15. The interactions between the factors and responses were identified by using quantitative data. After the statistical analysis, the optimized extraction conditions were obtained.

Table 1: Independent factors with their coded and actual values used for optimization of UAE

Independent factors	Symbol	Coded Levels		
		-1	0	1
Time (min)	A	10	20	30
Amplitude (%)	B	60	80	100
Ethanol concentration (%)	C	0	20	40

Purification of protein by Acetone

Acetone precipitation was done to purify the protein sample. Acetone was taken and cooled to (-20 °C). Protein sample obtained was added to the cold acetone four times the volume of the protein sample and vortexed thoroughly. Then the sample was incubated at - 20 °C for 60 minutes and then centrifugation at 13,000 g for a time interval 10 min. The supernatants after centrifugation were thrown and tubes which has pellet was in inverted direction for 15 min in order to remove acetone from the pellet which can be of excess. Re Dissolving of protein pellet was done in 0.01 mol/L phosphate buffer (pH 7.0). Then the solution was dialyzed against 0.01 mol/L phosphate buffer (pH 7.0) using a 1 kDa dialysis membrane for 14 h at 4 °C[11].

Proximate analysis

The dried pineapple (core and peel) powder was subjected to the determination of moisture content, Ash content, Volatile content and fixed carbon content using thermogravimetric analyzer (TGA 701, LECO) was used using AOAC method.

Quantitative analysis

Total Protein content

To determine the protein present in the sample Bradford standard was taken as reference and used in the sample determination. BSA reagent was made by diluting the reagent with distilled water in the ratio of 1:4.100 µl. and 5 ml of Bradford dye was added to the sample solution. Then it was vortexed thoroughly and mixture was kept for incubation at ambient room temperature for 15 min and then absorbance was measured at 595nm[3] using UV spectrophotometer (UV-UNICAM, ALVA,U.K).

Total phenolic content

Folin-Ciocalteau method [14] is a prominent method which was used to determine the amount of total phenolic present in the sample. The sample of 1 ml was taken and diluted with 9 ml distilled water, from there 0.5ml of the diluted sample was taken and added with folins reagent of 2 ml which was freshly prepared at 1:10 ratio. And to the mixture 7.5% sodium carbonate of 4 ml was added and then kept for incubation for a time duration 30 minutes long and using the UV spectrophotometer (UV-UNICAM, ALVA, U.K) the absorbance was measured at 765 nm.

Ferric reducing antioxidant power

Antioxidant activity of the crude extract sample was determined using the FRAP assay, using ferrous sulphate as the standard. Acetate buffer 300mM was used to prepare the frap reagent, then after 10 mM of TPTZ and 20 mM of ferric chloride was taken in the ratio (10:1:1). In 40 mM Hcl, 10 ml of 0.031 g TPTZ was dissolved in a water bath at 50°C. Ferric chloride was therefore dissolved in distilled water of 10 ml. Both were freshly prepared before use. To 100 µl of the sample, the FRAP reagent of 3 ml was mixed, vortexed and kept for incubation during a time period of 3 min and the absorbance was measured at 593 nm in the UV spectrophotometer (UV-UNICAM, ALVA, U.K)

DPPH radical scavenging activity

To determine the IC_{50} of the DPPH for optimized condition of the pineapple peel and core was done by DPPH assay[25]. A software known as Graph Pad prism (GPM6-1123456-ABCD-1234) was used to determine the IC_{50} value using the equation 1/ Y^2-weighted non-linear regression; log (inhibitor concentration) vs. normalized response model with a variable slope DPPH is known to be a stable radical because of its paramagnetic property that is conferred by the odd electron. Through this property it has the ability of color change in the solution from deep violet to yellow while it is prepared with ethanol. This change can be determined by the absorbance at 515 nm using UV spectrometer. The sample to be tested for antioxidant activity was added to DPPH solution and which results in a pale violet, showing the effect of antioxidant. For testing the sample was diluted for at least five concentrations (two-fold dilutions) i.e. 1000, 500, 250, 125 and 62.5µl. And $6*10^{-5}$ M of DPPH was prepared in absolute ethanol and then 1 ml of the sample was added with DPPH solution of 1 ml. The mixture was kept in the dark for 30 minutes incubation and measurement of absorbance was at 515 nm in the UV spectrophotometer (UV-UNICAM, ALVA, U.K)

$$\text{DPPH scavenging (\%)} = \frac{\text{Absorbance of control} - \text{Absorbance of sample}}{\text{Absorbace of control}} * 100$$

Electrophoresis Analysis: (Bio-Rad Laboratories, Inc., Richmond, CA, USA)

The molecular weight and protein pattern determination was determined using the SDS page.

Table 2: SDS-PAGE (Polyacrylamide gel electrophoresis) using the chemical formulation

Chemical formulation	Quantity
12% gel	4 ml
1.5 H Tris, pH 8.8	2.5 ml
0.5M Tris, pH 6.8	1.250 ml
Distilled water	3.345 ml
10% SDS	100 µl
10% APS	50 µl
TEMED	5 µl
Total volume	10 ml

The protein samples obtained from the optimized conditions of UAE was mixed with sample buffer and 15 µl was loaded into the gel and then it was subjected to electrophoresis (Bio-Rad Laboratories, Inc., Richmond, CA, USA)using the electrode buffer (glycine – 14.4 g, Tris – 3 g and SDS- 1 g which was made to 1 liter) at the current 15 mA/gel[13]. After when the separation was done the gel was put in a stainer (Coomassie Brilliant Blue) and was left with slight shaking for 12 h and then it was taken and put in a destainer. After activity, staining was done to substantiate the bromelain using casein- substrate gel. The gel was completely merged in 50 ml of 2% casein 50mM phosphate buffer, for 45 mins with constant agitation[9]. Then at 37 °C gel incubation was done for 30 minutes to discern a clear zone on background of blue, which helped in confirmed the bromelain activity.

Protease assay for bromelain activity

The determination of bromelain activity was by done by casein digestion method by the presence of cysteine and EDTA (Murachi 1976) using casein as a substrate. The assay was of proteolytic hydrolysis in which the bromelain breaks up the smaller peptide bonds present and helps in releasing the amino acids which are free after hydrolyzing it with protein. In this case L-tyrosine was released from casein after hydrolysis with bromelain enzyme. Unhydrolyzed substrate was precipitated using TCA. The assay was mainly based on colorimetric method[27].

In this assay, suitable vials (in millilitres) were taken and the reagent casein of 5 ml was pipette into the test and blank, therefore it was equilibrated to 37 °C and enzyme solution of 1 ml was added to it and mixed by swirling and was incubated for 10 minutes at 37 °C. Then after TCA of 5 ml was added to the test and blank followed by the added solution of 1 ml to the blank which was centrifuged and incubated at 37 °C for 30 minutes and filtered through a syringe filter and the filtrate was used in colour development. For the determination of the colour the test filtrate and the blank was taken 2 ml and then sodium carbonate 5 ml was added to both test and blank filtrate followed by folin reagent of 1 ml. Then it was centrifuged and cooled to room temperature and the absorbance was measured at 275 nm.

The enzyme activity was calculated using the equation:

$$\text{Activity (CDU/ml)} = Et - \frac{Eb}{Es} * \text{concentration of standard Ltyrosine} * \frac{Vr}{Tr} * Df$$

Where,

E_t - absorbance of enzyme sample; E_b - absorbance of enzyme blank; E_s - absorbance standard L- tyrosine; D_f - dilution factor; Vr - reaction volume; tr - reaction time.

RESULTS AND DISCUSSION

Proximate analysis

The proximate analysis of the pineapple peel and core was determined using the Thermo gravimetric analyser (TGA701, LECO) and the results were depicted.

Table 3: Proximate analysis of peel and core

Content	Proximate Analysis In percentage (%) w/w	
	Peel	Core
Moisture content	7.97± 0.05	7.29 ± 0.04
Volatile content	70.03± 0.37	72.22 ± 0.33
Ash component	3.47± 0.06	1.30 ± 0.02
Fixed carbon	18.51± 0.39	19.18 ± 0.32

**Results are in means ± SD*

The moisture content of the peel and core was found to be less and therefore the products with low moisture content, are not much subject to the effect of degradation by microbes and other chemical changes that occurs within the products.

Table 4: Range of Bioactive present obtained from the Response surface analysis

Treatment	Sample	Quadratic model	Protein *µg/g*	TPC *µg of GAE/g*	FRAP *µmol/g*
Ultrasound Extraction	**Peel**		2979.4 -4526.82	3505.19 - 8208.36	18.55 - 153.02
		R^2	0.7136	0.8406	0.7763
	Core		3581.69 - 6612.83	9340.26 - 24678.7	349.93 – 502.3
		R^2	0.6945	0.7091	0.7930

The volatile compounds are the ones that contribute to the aroma and the flavour as they are comprised of many compounds such as esters, alcohols, aldehydes, ketones, lactones, terpenoids and apocarotenoids. They also play special functions and also help the plant in preventing oxidative stresses. Both the peel and core has higher volatie content. The ash content of the sample determines the amount of minerals present, therefore accordingly the pineapple core has less minerals than the peel.

Model fitting

Response surface analysis was used as a tool to optimize the extraction conditions for UAE treatment and using the responses, bioactive components were also analysed. The overall experimental design gave 15 runs and therefore the regression analysis was also done for curve fitting for the quadratic model and the significant difference from ANOVA was also obtained. Models summary statistics showed that the cubic models were found to have R^2 values between 0.80 to 0.99, indicating the regression models were suitable to explain the behavior of the model. In addition, the adj-R2 values (cubic model) for the total phenolic content and FRAP of the peel were found to be higher than 0.910, indicating that insignificant term have not been included in the model. However, the cubic model was found to be aliased signifying that the effects of each variable caused different signal to become indistinguishable[24].

Extraction of bioactive active components from peel of pineapple

The range of protein, TPC and FRAP given in Table 4. The protein content differs according to the purification and extraction techniques used. By means of membrane processing the protein content was found to (1.37 mg/ml)[21]. Therefore, the protein content of the peel differs in every cultivars. The major polyphenols present in the pineapple peel are the catechin, epicatechin, ferulic acid and gallic acid Therefore phenolic content of the peels was found to be 31.98 mg gallic acid equivalents (GAE)/g extracts[15]. In regard with TPC[6] 9.1 mg GAE/g dry TPC of pineapples and pineapple residues were found to be 2.75 mg GAE/g dry weight (DW). The other study[28] showed that pineapple peel was non-concentrated with soy flour (1249 µg/g

– 170.93 µg/g) and further concentrated (14691.5 µg/g – 2788.6 µg/g) which was comparatively found to possess less phenolic content from this present study of the UAE treatment. Ferric reducing power assay shows the ability of the extract to donate electron to ferric ion, reducing it into ferrous ion. The ferulic acid present in the pineapple peel mainly contributed to the antioxidant[31]. FRAP value was found to be (6.9µM TE/g) and (2.5 µM TE/g) using solvent such as methanol: acetone and ethanol respectively[17]. The effect antioxidant activity may differ according to the type and complexity of the sample used for study.

The ANOVA was also performed to get the significant difference between each variable on the protein, phenolic content and antioxidant from the extraction process. It showed that in case of protein time, amplitude, ethanol concentration used was not significant, and as the value for the model term was greater than 0.100 which showed it wasn't significant. In extraction of TPC, there was a significant difference in the amplitude ($p<0.05$) where time and ethanol concentration has no significant difference. In the determination of antioxidant activity, there was no significant difference shown between the variables used and so is the model. But the lack of fit for the model is significant. The optimised condition obtained was time 10 min, 100% amplitude and ethanol concentration 28% gave 4166.11 µg/g 8054.77 µg GAE/g 124.7388 µmol Fe(II)/g with desirability 0.837.

In the extraction process the interaction between the independent variable and the responses Time (A), Amplitude (B) and ethanol concentration (C) are given as multiple regression equation which represents an empirical relation is given below:

$$Y_1 = 4041.51 + 183.137\,A + 320.011B + 165.169\,C - 248.517\,AB + 129.992\,AC - 10.705\,BC - 101.062\,A^2 - 159.175\,B^2 - 62.065C^2 \quad \ldots(1)$$

$$Y_2 = 6264.88 - 243.705\,A + 1180.41B + 542.853C - 600.603AB + 177.873AC + 542.853BC - 239.858\,A^2 - 251.408B^2 - 699.549\,C^2 \quad \ldots(2)$$

$$Y_3 = 35.5567 - 13.68A + 19.4075B + 4.4825C - 32.175AB - 2.075AC + 1.68BC \quad \ldots(3)$$

Here Y_1, Y_2, Y_3 represent the protein content, phenolic content and FRAP content of peel and A, B, C are the actual values of the independent variables.

The optimised condition and the three-dimensional response graph was generated in which one variable is fixed and other two were varied to show the interactive effects. The plots are show in Fig. 1, 2 & 3.

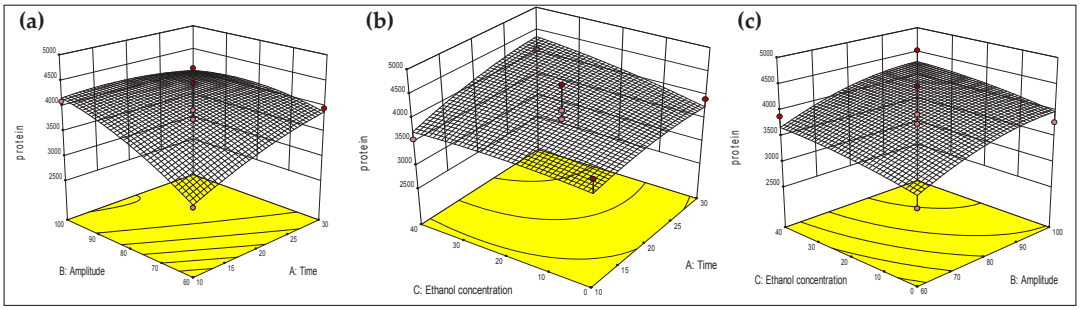

Fig. 1: Response surface plots showing effect of time (min), amplitude (%) and ethanol concentration (%) on protein content (µg/g of crude extract) from pineapple peel using UAE treatment (a) Time & amplitude (b) Ethanol concentration & time (c) Ethanol concentration & amplitude

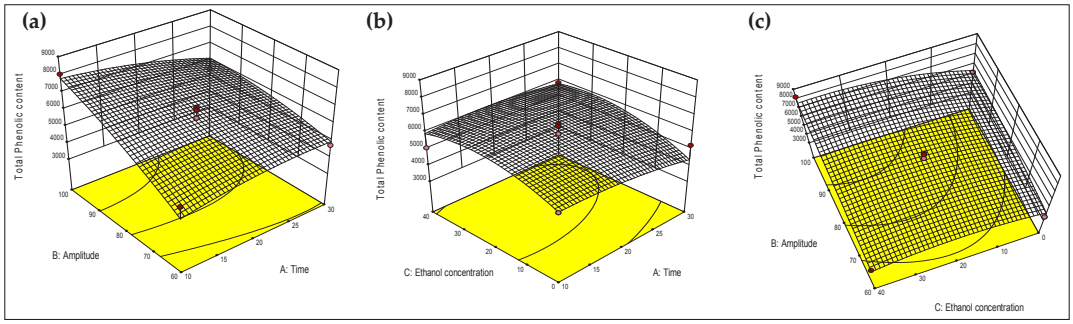

Fig. 2: Response surface plots showing effect of time (min), amplitude (%) and ethanol concentration (%) on phenolic content (µg of GAE/g) from pineapple peel (a) Time & amplitude (b) Ethanol concentration & time (c) Ethanol concentration & amplitude

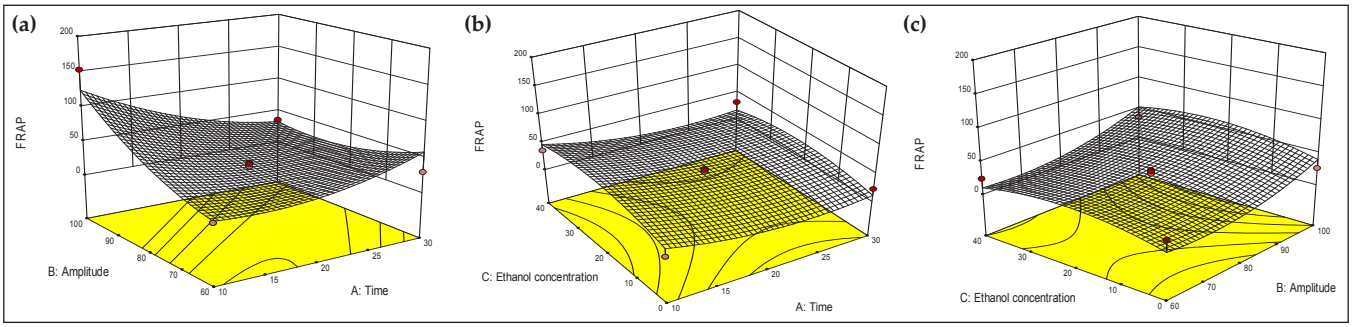

Fig. 3: Response surface plots showing effect of time (min), amplitude (%) and ethanol concentration (%) on antioxidant content (µg of GAE/g) from pineapple peel (a) Time & amplitude (b) Ethanol concentration & time (c) Ethanol concentration & amplitude

Extraction of bioactive active components from core of pineapple

In this study under UAE from the Table 4, the protein content ranged from 3581.69 – 6612.83 µg/g the core of the *Nang Lae* pineapple has a protein content of 45.4mg/100g and *Phu Lae* has a 27.1mg/100 g[12]. The protein content depends on the extraction buffer ph, temperature and strength of extraction buffer. Therefore the protein content was found to be 12.6mg/ml[4]. From Table 4, tpc content was 9340.26 – 24678.7 µg GAE/g. Extracting the phenolic content in the core by using ethanol as solvent was nearly 10mg/g GAE[30]. Therefore from our study UAE treatment gave comparatively higher phenolic content. This is because UAE uses sound waves at frequencies above the range audible to humans (=20 kHz) to disrupt the plant cell wall thereby enhancing solvent penetration into the plant material and facilitating the release of extract and giving higher extraction efficiency[16]. The FRAP value of the pineapple core was found to be 2.01 mmol/100[10]. In our study the FRAP value ranged from (349.93 – 502.3 µmol Fe(II)/g. The ferulic acid which is most abundant hydroxycinnamic acid is present in the plant cell walls has contributed a lot to the antioxidant activity.

The ANOVA portrayed that there was no significant difference on each variable such the time, amplitude and ethanol concentration on the protein, phenolic content and antioxidant content from the extraction process, and so is the model and the lack of fit as ($p>0.05$). The optimised condition of core for the maximum yield of protein 4860.58 µg/g, TPC 20192.19 µg GAE/g and 438.60 µmol Fe(II)/g for Time 16(min), amplitude 100%, ethanol concentration 36%.

Therefore, the predicted equation shows an empirical relation between the variables and responses is obtained using multiple regression equation

$$Y_4 = 4084.33 - 63.4663\,A - 295.161B - 252.34C - 391.507AB - 108.585AC + 792.195BC - 124.384\,A^2 - 215.126\,B^2 - 325.239C^2 \quad \ldots(4)$$

$$Y_5 = 20565.3 + 1648.76A + 2784.71B + 1116.31\,C + 738.046\,AB - 612.15\,AC + 1999.3\,BC - 1738.66A^2 - 2910.99B^2 - 2835.91\,C^2 \quad \ldots(5)$$

$$Y_6 = 425.493 - 26.5175A + 33.0587\,B - 14.8438C + 17.4375AB + 8.6125\,AC - 9.445BC - 11.9367\,A^2 - 17.5408B^2 - 23.2492 * C^2 \quad \ldots(6)$$

Here Y_4, Y_5, Y_6 represent the protein content, phenolic content and FRAP content of core and A, B, C are the actual values of the independent variables.

Determination of DPPH radical scavenging activity of the pineapple peel and core

The antioxidant capacity is a significant indicator as health promoter in many fruits and vegetables peels. It's widely used to detect antiradical activity of different samples, due to its sensitivity to lower concentrations of active principles from natural source. Different concentration of each sample (1000 µg/ml, 500 µg/ml, 250 µg/ml and 125 µg/ml) was prepared and the % inhibition was determined. The IC50 value was determined using the 1/Y2-weighted non-linear regression; log (inhibitor concentration) vs. normalized response model with a variable slope. The Data analysis was obtained using Graph Pad Prism® version 6.01 (San Diego, US) is given in table below. The lower IC50 value exhibits a higher antioxidant potential, in our study the pineapple core has lower IC_{50} value of 38.65 µg/ml which depicts higher antioxidant potential is present in the core compared to the peel.

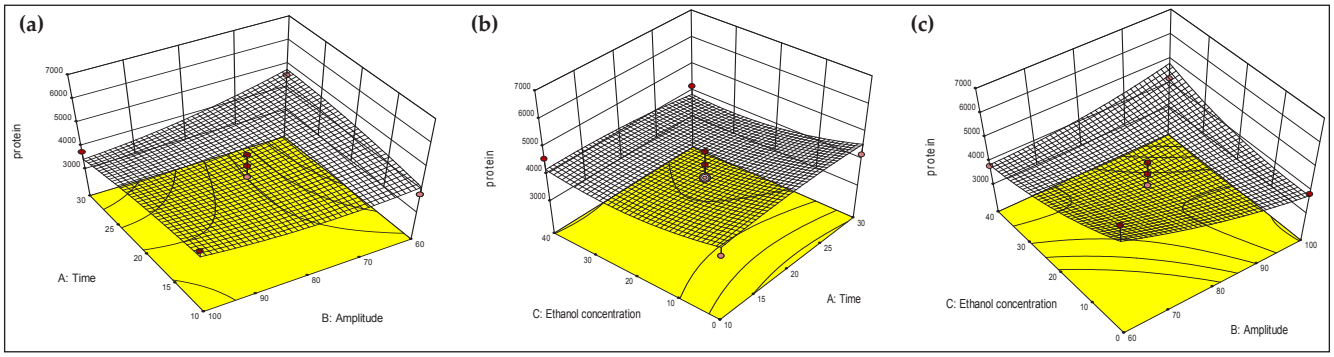

Fig. 4: Response surface plots showing effect of time (min), amplitude (%) and ethanol concentration (%) on protein content (µg/g of crude extract) from pineapple core (a) Time & amplitude (b) Ethanol concentration & time (c) Ethanol concentration & amplitude

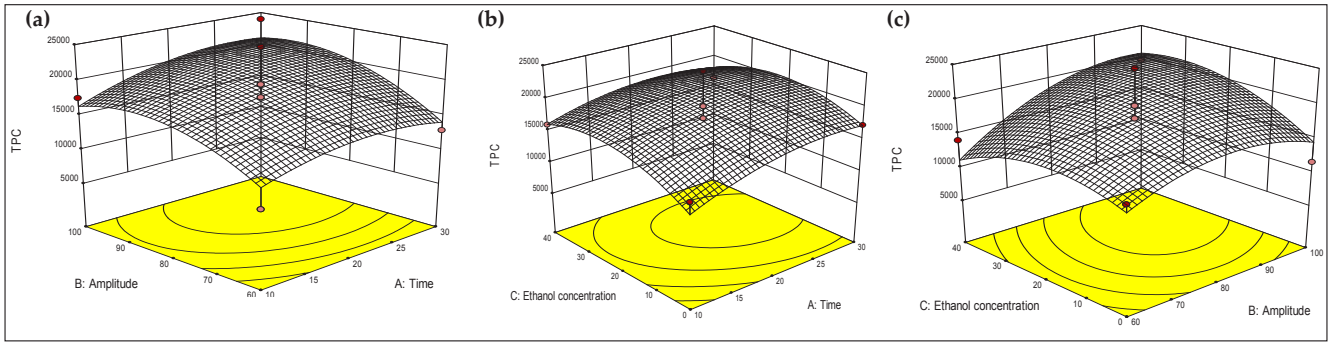

Fig. 5: Response surface plots showing effect of time (min), amplitude (%) and ethanol concentration (%) on phenolic content (µg of GAE/g) from pineapple core using UAE treatment (a) Time & amplitude (b) Ethanol concentration & time (c) Ethanol concentration & amplitude

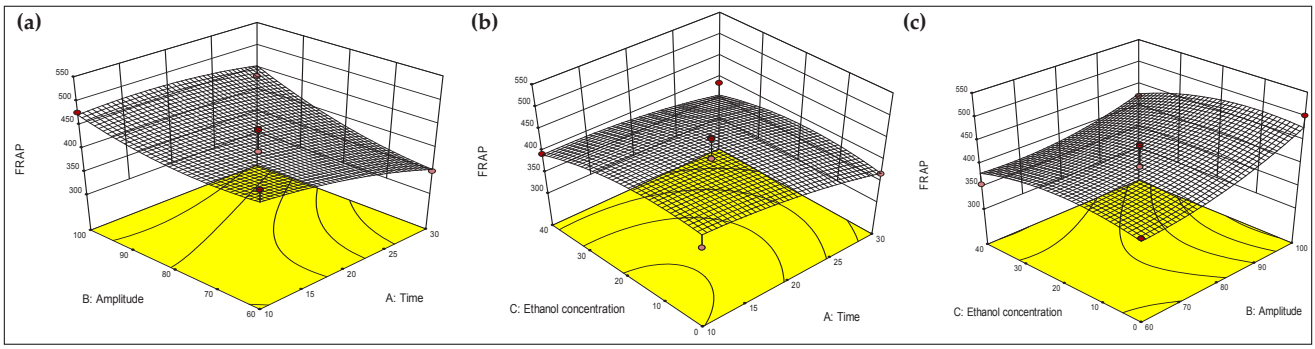

Fig. 6: Response surface plots showing effect of time (min), amplitude (%) and ethanol concentration (%) on antioxidant content (µg of GAE/g) from pineapple core using UAE treatment (a) Time & amplitude (b) Ethanol concentration & time (c) Ethanol concentration & amplitude

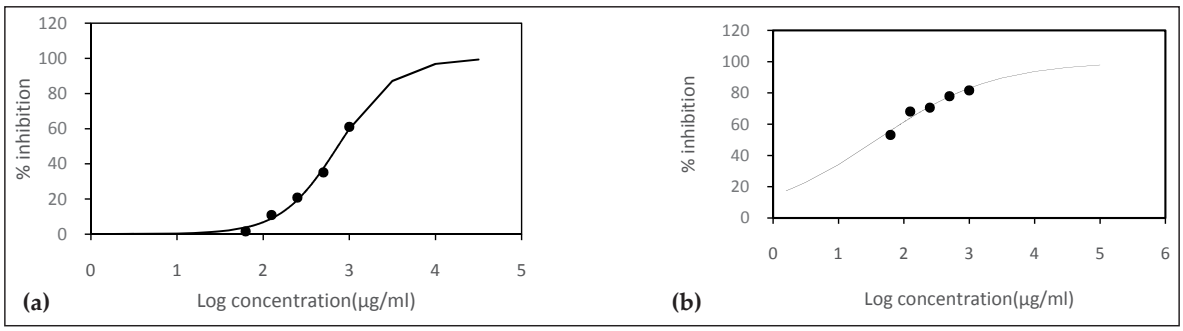

Fig. 7: Estimated DPPH inhibition of (a) peel and (b) core of pineapple at different concentrations, using nonlinear regression

UAE treatment is more significant in determining the antioxidant capacity this because of the strong disruption of the cells which helps in squeezing out the bioactive compounds from the cells efficiently. The estimated DPPH inhibition of peel and core of pineapple at different concentrations, using nonlinear regression was depicted in the form of graph.

Table 5 : IC_{50} value of peel and core

UAE treatment of sample	IC 50 (µg/ml)	Hill slope	R^2
peel	738.3	1.311 (0.9870 - 1.634)	0.9918
core	38.65	0.4873 (0.2563 -0.7182)	0.9447

Determination of Bromelain activity

Bromelain activity was determined using casein digestion method. From our study it was found that, after purification with acetone and dialysis the bromelain activity and purification fold is given in Table 6. The enzyme activity was much dependent on the extractant solvent used and different varieties of pineapple gave different results[12] therefore for the distilled water as solvent NL variety of peel gave a activity of 327.71(units/ml) the same PL variety gave 443.66 units/ml. From our study using ethanol as extracting solvent the activity of peel and core was found to be 131 ± 0.025 units/ml and 116.25 ±0.405 units/ml with a fold purification of 1.25 and 1.17 respectively. The bromelain activity depends on the extraction buffer, temperature and the purification techniques used, from the study of[4] the activity of core bromelain ranged from 155 – 130 cdu/ml depending on the temperature 5 to 40 °C. And therefore the purification fold was in the range (2-5 folds) depending on the concentration of acetone used.

Protein patterns

The molecular weight of the protein of the crude waste mixture which contains (57% peel, 28%

Table 6: Bromelain activity for the optimised condition of the pineapple peel and core

	Before purification			After purification			
	Protein (mg/ml)	Bromelain activity (unit/ml)	Specific activity (unit/ml)	Bromelain activity (units/ml)	Protein (mg/ml)	Specific activity (unit/ml)	Fold purification
UAE peel	4.25± 0.005	94.20 ±0.05	22.14	131 ± 0.025	4.716 ± 0.022	27.78	1.25
UAE core	3.65 ± 0.009	93.13 ± 0.005	25.61	116.25 ±0.405	3.886 ± 0.043	29.85	1.17

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 after purification

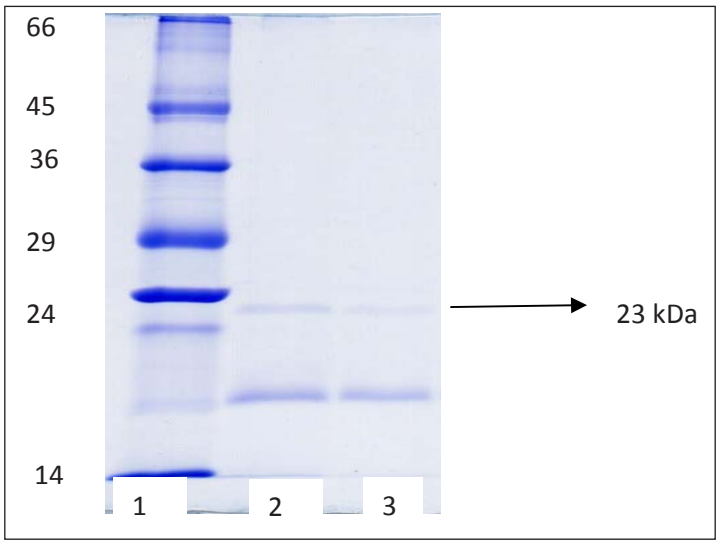

Fig 8: SDS page analysis: Lane 1 – Ladder; Lane 2 – UAE peel; Lane 3 – UAE core

crown and 15 % core) has between 11.7 and 26.9 kDa[21]. The protein pattern of the peel from different cultivars ranged from 23- 28 kDa[12]. From the Fig. 8, the protein pattern was found to be in the range of 23 kDA for both peel and core. Therefore, the activity staining for the proteolytic activity was positive and confirmed the presence of bromelain.

CONCLUSION

The Box–Behnken Design (BBD) was demonstrated to be an effective and reliable technique for investigating the extraction of bioactive compound.

The bromelain activity for the optimized condition for the peel and core was found to be 94.20 ±0.05 (unit/ml) and 93.13 ±0.005 (unit/ml) for the pineapple peel and core respectively. Therefore, after purification, 131 ± 0.025 units/ml and 116.25 ±0.405 with specific activity of 27.78 and 29.85 with fold purification 1.25 and 1.17. The molecular weight of protein in both peel and core was found to be 23 KDa. Staining was done to confirm the presence of bromelain which depicted the result to be positive. I C_{50} value proved that core had higher antioxidant potential than the peel. Therefore from our study we can conclude that UAE is one of the quickest method for obtaining the bioactives and the obtained bromelain can be applied in meat tenderization, baking process and also it can be used in various pharmaceutical industry.

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