Utilization Of Unripe Papaya Peel Waste (Carica Papaya) And Banana Peel Waste (Musa Acuminata) For The Extraction Of Bromelain Enzyme And Its Optimization

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ABSTRACT

Bromelain being the major protease enzyme abundantly seen in fruits like pineapple, banana, Kiwi, papaya has wide range of applications in process industries, pharmaceutical industries, food, cosmetic, leather industries, recovery of metals, etc. In this study, papaya peel waste and banana peel waste are used as raw materials for the extraction of bromelain enzyme. This study includes the optimization of parameters such as temperature of solution, incubation time, PH of solution and concentration of solution for both the resources (Wastes of banana peel and papaya peel). Response surface methodology was used to analyze the optimum conditions using box behnken design for bromelain extraction. The optimum conditions using papaya peel waste are observed at 40minutes of incubation time, pH 5.0, 50g/L of crude bromelain extract at 60℃ solution temperature yielding 84.67% of bromelain activity with gelatin as substrate; whereas for banana peel waste maximum yield of 93.35% was seen at 30minutes of incubation time, pH of 5.0, with 50g/L of crude bromelain extract (from Banana peel waste) maintained at 50℃ of solution temperature. The enzyme activity was measured using tyrosine as standard and expressed in µg/mL.

Characterization like XRD, SEM and FTIR were performed for both the papaya and banana sources before and after the extraction of bromelain enzyme.

Keywords: Bromelain enzyme, papaya peel waste, banana peel waste, response surface methodology, tyrosine.

1. INTRODUCTION

Besides many applications of bromelain enzyme in sectors like food[1], cosmetic[2], textile[3], leather[1], medical, pharmaceutical[4] and other industries; wide research is still taking place in studying and optimizing the enormous potential of bromelain enzyme activity, sources, extraction techniques.

Bromelain is a natural complex mixture of various enzymes containing cysteine amino acid side chain[14] which is a combination of different thiol endopeptidases and other constituents like cellulose, glucosidase and other inhibitors[14]. Majorly bromelain is present in fruits, stem and leaves. The more percentage of bromelain content is present in pineapple plant.

Muhammed [5] utilized pineapple peel waste as a source for the extraction of bromelain which was further utilized for the recovery of Silver from X-Ray films. In his study, the Bromelain enzyme was repeatedly used more than 50 times without the loss of its activity and observed that the enzyme was heat tolerant since they have utilized bromelain for the extraction of silver from X-Rays at a temperature of 70℃. Resmi Mohan[6] et al also worked on the extraction of bromelain from pineapple source where both fruit and the peel were utilized. The extracted bromelain was used in the process industry for unhairing and leather making applications. Erku [7] et al with the help of process optimization worked on the recovery of silver from waste X-ray films, yield of 1.07% w/w was obtained at 70.88℃, 10.97min and 1.5M NaOH concentration and Addis and Omprakash [8] used Resonance Surface Methodology for the optimization of silver recovery from waste radiographic film.

Novaes [9] et al used pineapple peel waste for the extraction of bromelain by polymer-based method by achieving the yield of 335.27% with a purification factor of 25.78.H. Umesh Hebbar [10] et al investigated on the

utilization of reverse micellar systems for the extraction and purification of bromelain from pineapple wastes by which forward and backward extraction efficiencies of 45% and 62% were obtained using CTAB system. Sunanda Ketnawa [11] et al worked on the aqueous 2-phase extraction of bromelain from pineapple peels and the bromelain showed the highest relative activity at pH 8 and 60°C. Gautam S. S. [12] et al worked on the extraction of bromelain form pineapple plant stem and fruit. Sunantha Ketnawa [13] et al investigated on the bromelain extraction from pineapple peel wastes, pineapple peel, core, stem, crown was utilized as sources and it was observed that the relative activity of all the sources are >80%.

As per the literature there is no research in the usage of unripe papaya peels and banana peels as source for the extraction of Bromelain and its optimization. Hence, in the present study, the main objective was designed to utilize the waste for the effective recovery and usage of a widely used enzyme like bromelain. However, the current techniques used for the recovery of bromelain utilizes minimum amounts of chemicals and do not pose any burden to the environment.

2. MATERIALS AND METHODS

2.1. Unripe papaya peel source: Unripe papaya fruits were collected from the neighbourhood of Kurmannapalem in Visakhapatnam. These fruits were thoroughly washed, dried, and peeled; the peels were carefully dried at room temperature without exposing to the sun[22], these peels were ground into powder and stored in a refrigerator at 5°C. Fig.1 (a) represents the process from collection of unripe green papayas to powder preparation.

2.2. Banana Peel source: Bananas were collected from the local market of Pedaravuru in Tenali. Bananas were thoroughly washed; fruits were utilised and the peels what carefully dried at room temperature. The dried Peels were verified for the presence of moisture, after complete drying the peels were ground into powder which were further stored in a refrigerator at 5°C. Fig.1 (b) shows the cycle of conversion of banana waste peels to Banana powder source.

Fig.1. (a) Process of conversion of unripe Papaya peel waste to powder source

Fig.1. (b) Process of conversion of Banana peel waste to powder source

2.3. Chemicals & Equipment Utilized during the Study:

Sodium carbonate, Hydrochloric acid, Sodium hydroxide, 10% Trichloroacetic acid, 1% Gelatin, Phosphate buffer, 2N Folin & Ciocalteu Phenol Reagent, Tyrosine, chemicals were purchased from Sree Sai Enterprises Pvt. Ltd. Visakhapatnam. The types of equipment used for the present study are pH meter, Mixer, Centrifuge, Digital Water Bath, Magnetic Stirrer, Heating Mantle, UV Visible Spectrometer, Weighing balance.

2.4. Extraction of Crude Bromelain Enzyme:

Powdered samples of Papaya and Banana were mixed with DI water in a ratio of 1:20 and then the juice was heated on a heating mantle with the help of a magnetic stirrer for 30min at 50°C, the solution was allowed to reach room temperature and then filtered using Whatman filter paper. The filtrate was centrifuged at 5000 rpm at room temperature and the supernatant was stored in a refrigerator and used for further experimentation. The solutions obtained from respective sources were used as crude bromelain enzymes from Papaya peel and banana peel wastes.

2.4.1. Bromelain Enzyme Assay from Papaya Peel Waste & Banana Peel Waste:

Step 1: Bromelain enzyme activity was determined using gelatin as substrate[5]. 500µL of Crude enzyme(Papaya & Banana – Studies were performed separately) was mixed with 500 µL of gelatin and incubated for varying time (Parameter A), at a known pH (Parameter B), known temperature of solution (Parameter C) at known concentration (Parameter D).

Step 2: Post incubation phenomenon, 500µL of 10% TCA was added to the solution along with 500µL of crude bromelain enzyme and left undisturbed for 20 minutes at room temperature.

Step 3: After 20min, the solution was centrifuged for 18 minutes at 5000 rpm at room temperature.

Step 4: Post Centrifugation, 500µL of Supernatant was taken into a beaker, to which 500µL of 2N Folin & Ciocalteu Phenol Reagent diluted in 1:10 ratio was added along with 2.5mL of Na_2CO_3 solution. The solution was allowed to swirl (Vortex formation) for 20 minutes at room temperature.

Step 5: Absorbance was measured for the executed samples in Ultraviolet Visible Spectrophotometer at 660nm. The enzyme activity was calculated using tyrosine standard calibration curve.

2.4.2. Optimization of Parameter A (Step 1) – Effect of Incubation Time:

A 50mL beaker was taken into a digital water bath which is contained with 500µL of Crude enzyme (Papaya and Banana Bromelain enzymes) and 500 µL of gelatin maintained at a pH of 4, concentration of 30g/L at 40°C. At varying incubation[20,21] time like 10, 20 ,30, 40 and 50 minutes, samples were collected, and further steps were followed in

sequence from Step 2 to Step 5. It was observed that the optimum incubation time was observed at 40 minutes for papaya source and 30 minutes for Banana Source.

2.4.3. Optimization of Parameter B (Step 1) – Effect of pH of Solution:

500µL of Crude enzyme (Papaya & Banana Bromelain Enzymes) and 500 µL of gelatin were taken into a beaker kept in a water bath maintained at a concentration of 30g/L at 40℃ and optimum incubation time of Bromelain enzyme obtained from Papaya and Banana sources were maintained respectively for the bromelain studies obtained from papaya and banana sources. At varying pH like 4,5,6 and 7 samples were collected, and further steps were followed in sequence from Step 2 to Step 5. Post analysis in UV Visible Spectrophotometer, optimum pH was found to be 5 for both the bromelain enzymes obtained from Papaya and Banana sources.

2.4.4. Optimization of Parameter C (Step 1) – Effect of Temperature of Solution:

500µL of Crude enzyme (Papaya & Banana Bromelain Enzymes) and 500 µL of gelatin were taken into a beaker which was maintained in a digital water bath at a concentration of 30g/L, optimum pH of 5, and optimum incubation time of Bromelain enzyme obtained from Papaya and Banana sources were maintained. At varying solution temperatures like 30℃, 40℃, 50℃, 60℃, 70℃ and 80℃; samples were collected, and Steps from 2 to 5 were followed. Post analysis in UV Visible Spectrophotometer, optimum solution temperature was found to be 60℃ for Papaya and 50℃ for Banana Bromelain sources.

2.4.5. Optimization of Parameter D (Step 1) – Effect of Concentration of Solution:

Stock solutions were prepared at 10g/L, 20g/L, 30g/L, 40g/L, 50g/L and 60g/L concentrations for both papaya and banana sources. 500µL of Crude enzyme (Papaya & Banana Bromelain Enzymes) at varying concentrations and 500 µL of gelatin were taken into a beaker which was maintained in a digital water bath at optimum temperatures of both the enzymes, bromelain from papaya maintained at 60℃ and bromelain from banana maintained at 50℃, optimum pH of 5, and optimum incubation time of Bromelain enzyme obtained from Papaya(40minutes) and Banana(30minutes) sources were maintained. At varying solution temperatures like 30℃, 40℃, 50℃, 60℃, 70℃ and 80℃; samples were collected, and Steps from 2 to 5 were followed. Post analysis in UV Visible Spectrophotometer, optimum concentration was found to be 50g/L for both Papaya and Banana Bromelain sources..

2.4.6. Preparation of Tyrosine Standard Curve:

0.5M Na_2CO_3, 2N Folin & Ciocalteu Phenol Reagent diluted in 1:10 ratio, distilled water and 1mg/mL of Tyrosine stock solution were used for standard curve preparation. Varying concentrations of Tyrosine were considered in each test tube to which 2.5mL of Na_2CO_3 and 500µL of 2N Folin & Ciocalteu Phenol Reagent were added into each test tube and distilled water was added into each test tube including blank. Solutions were thoroughly mixed for 30 minutes at room temperature. Absorbance was measured using UV Visible Spectrophotometer at 660nm. To measure the crude bromelain activity calibration curve was generated following the regression equation $y = 0.0102X + 0.0206$ with $R^2 = 0.9977$.

2.5. Design of Experiments:

Response Surface Methodology was opted for designing and optimizing the parameters. In RSM, Box Behnken Design was chosen to explore the relationship between response or % y Yield (Bromelain Enzyme activity) and the 4 parameters namely Incubation Time(X1, minutes), pH of solution(X2), Temperature of solution(X3,℃) and Concentration of solution(X4, g/L). In total 27 experimental runs were performed by considering all the 4 factors X1, X2, X3 and X4 at Low, Medium and High levels. Table.1. represents the range and levels using for optimization of Bromelain activity from papaya and banana peel sources. Minitab 20.2 (64-bit) statistical software was used for the analysis. Table.2, Table.4 indicate the Bromelain enzyme recovery on combination of parameters from Papaya and Banana sources whereas Table.3, and Table.5. indicate the Analysis of Variance for Bromelain activity of papaya and banana sources respectively.

Table.1. Parameters, Range and Levels (Common for both the Bromelain enzymes obtained from Papaya and Banana Sources)

Parameters	Range	Low (-1)	Medium (0)	High (+1)
Incubation Time, minutes	10 – 50minutes	10	30	50
pH of solution	4 - 7	4	5.5	7
Temperature of solution, ℃	30℃ – 80℃	30	55	80
Concentration of solution, g/L	10g/L – 60g/L	10	30	60

Table.2. Bromelain enzyme recovery on combination of parameters from Papaya source

Run No.	X1(min)	X2	X3(°C)	X4(g/L)	% y(Papaya)
1	30	4.0	55	10	10.1670
2	30	4.0	30	35	32.8501
3	30	5.5	80	10	20.9258
4	30	4.0	55	60	54.8159
5	30	7.0	80	35	38.9467
6	10	5.5	55	60	52.1262
7	10	7.0	55	35	51.8572
8	30	5.5	55	35	57.0573
9	50	5.5	55	60	84.6715
10	30	5.5	80	60	56.6090
11	10	5.5	55	10	34.2846
12	50	5.5	30	35	48.6296
13	30	5.5	55	35	51.8572
14	10	5.5	80	35	32.4915
15	30	5.5	30	10	28.0983
16	50	4.0	55	35	46.0296
17	50	7.0	55	35	49.7951
18	10	5.5	30	35	55.9814
19	30	7.0	30	35	49.7951
20	10	4.0	55	35	27.1121
21	50	5.5	55	10	22.8086
22	30	5.5	55	35	51.8572
23	30	7.0	55	60	63.0643
24	30	4.0	80	35	28.5466
25	50	5.5	80	35	56.7883
26	30	5.5	30	60	68.8919
27	30	7.0	55	10	29.0845

Table.3. Analysis of Variance for Bromelain activity (derived from Papaya)

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Model	14	7197.23	514.09	213.98	0.000
Linear	4	5627.74	1406.94	585.62	0.000
X1	1	250.89	250.89	104.43	0.000
X2	1	574.38	574.38	239.08	0.000
X3	1	207.82	207.82	86.5	0.000
X4	1	4594.64	4594.64	1912.45	0.000
Square	4	678.88	169.72	70.64	0.000
X1*X1	1	0.00	0.00	0.00	0.987
X2*X2	1	538.97	538.97	224.34	0.000
X3*X3	1	160.83	160.83	66.94	0.000
X4*X4	1	113.7	113.7	47.33	0.000
2-Way Interaction	6	890.61	148.43	61.78	0.000
X1*X2	1	110.04	110.04	45.8	0.000
X1*X3	1	250.41	250.41	104.23	0.000
X1*X4	1	484.47	484.47	201.65	0.000
X2*X3	1	10.71	10.71	4.46	0.056
X2*X4	1	28.46	28.46	11.84	0.005
X3*X4	1	6.53	6.53	2.72	0.125
Error	12	28.83	2.4		
Lack-of-fit	10	10.8	1.08	0.12	0.993

Pure Error	2	18.03	9.01
Total	26	7226.06	

As per the analysis of independent and dependent variables, quadratic equation was obtained for the activity of bromelain enzyme from papaya source.

Table.4. Bromelain enzyme recovery on combination of parameters from Banana source

Run No.	X1(min)	X2	X3(°C)	X4(g/L)	% y(Banana)
1	30	4.0	55	10	20.2976
2	30	4.0	30	35	31.6179
3	30	5.5	80	10	23.5226
4	30	4.0	55	60	80.5849
5	30	7.0	80	35	28.6562
6	10	5.5	55	60	89.2726
7	10	7.0	55	35	35.3036
8	30	5.5	55	35	69.0671
9	50	5.5	55	60	90.9838
10	30	5.5	80	60	93.3531
11	10	5.5	55	10	25.5629
12	50	5.5	30	35	41.7535
13	30	5.5	55	35	68.4089
14	10	5.5	80	35	34.0531
15	30	5.5	30	10	32.3419
16	50	4.0	55	35	33.724
17	50	7.0	55	35	45.505
18	10	5.5	30	35	50.9019
19	30	7.0	30	35	51.1652
20	10	4.0	55	35	38.1995
21	50	5.5	55	10	31.7495
22	30	5.5	55	35	69.0671
23	30	7.0	55	60	86.9032
24	30	4.0	80	35	43.8596
25	50	5.5	80	35	57.4177
26	30	5.5	30	60	92.6950
27	30	7.0	55	10	26.4185

Table.5. Analysis of Variance for Bromelain activity (derived from Banana)

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Model	14	15409.9	1100.7	302.73	0.000
Linear	4	11801.6	2950.4	811.45	0.000
X1	1	64.6	64.6	17.76	0.001
X2	1	54.9	54.9	15.10	0.002
X3	1	32.1	32.1	8.82	0.012
X4	1	11650.1	11650.1	3204.12	0.000
Square	4	2960.8	740.2	203.58	0.000
X1*X1	1	771.9	771.9	212.3	0.000
X2*X2	1	1836.1	1836.1	504.99	0.000
X3*X3	1	665.0	665.0	182.91	0.000
X4*X4	1	44.1	44.1	12.14	0.005
2-Way Interaction	6	647.5	107.9	29.68	0.000
X1*X2	1	53.9	53.9	14.81	0.002

X1*X3	1	264.3	264.3	72.68	0.000
X1*X4	1	5.0	5.0	1.38	0.263
X2*X3	1	301.9	301.9	83.03	0.000
X2*X4	1	0.0	0.0	0.0	0.960
X3*X4	1	22.5	22.5	6.18	0.029
Error	12	43.6	3.6		
Lack-of-fit	10	43.3	4.3	30.02	0.033
Pure Error	2	0.3	0.1		
Total	26	15453.5			

As per the analysis of variables, the interdependency was explained by the quadratic equation obtained for the activity of bromelain enzyme from Banana source.

3. Results and Discussion

3.1. Bromelain activity from Papaya source:

By experimentation, as per the mentioned parameters, by maintaining pH at 4, concentration at 30g/L, Temperature at 40℃, varying incubation times were analysed using UV Visible Spectrophotometer and optimum Bromelain activity was seen at 40minutes of incubation time beyond which the activity remained stable. At 40minutes of incubation time, and by putting the parameters like concentration at 30g/L and Temperature at 40℃, pH of solution was optimized and the optimum value was found to be at 5 where the bromelain activity was more. At optimum incubation time and pH, solution maintained at 30g/L concentration, Temperature of solution was varied for which the optimum temperature is seen at 60℃. Maintaining optimum incubation time, pH, Temperature, concentration of solution was varied and optimized at 50g/L which is shown in Fig.2.(a-d) Higher Bromelain activity was observed at Incubation time of 40min, pH of 5, Temperature of solution at 60℃ and concentration of solution maintained at 50g/L.

Interactions between the variables and optimum levels for maximum response was shown in Fig.3.(a-f). From RSM using Box Behnken design for optimization of parameters, the model F-value of 213.98 indicated that the model is significant and P-Value is observed to be <0.05 for model which indicates the parameters and the terms are significant. The "Pred R-Squared" of 0.9858 is reasonable when "Adj R-Squared" is observed to be at 0.9914. Based on the parameters and the significant factors in ANOVA, the coefficients were estimated for the model and the regression equation in coded factors is given as follows:
Note: Bromelain activity and yield are used interchangeable.

$$Bromelain\ Activity(Papaya\ Source) = 53.591 + 4.572X1 + 6.918X2 - 4.162X3 + 19.567X4 + 0.011X1^2 - 10.053X2^2 - 5.491X3^2 - 4.617X4^2 - 5.245X1X2 + 7.912X1X3 + 11.005X1X4 - 1.636X2X3 - 2.667X2X4 - 1.278X3X4. \quad (1)$$

From Eq.1. as positive and negative coefficients were found for some interactions, positive coefficients maximize the activity whereas negative coefficients minimizes the activity of bromelain. We can infer that the increase in incubation time, pH and concentration would ultimately result in optimum bromelain activity, however increasing temperature beyond a certain level would reduce the activity of bromelain. Some interactions and square of these factors have inverse relation with the activity of bromelain. The effect of temperature of solution and pH are observed to be the prominent parameters for bromelain extraction from RSM and experimental results as well.

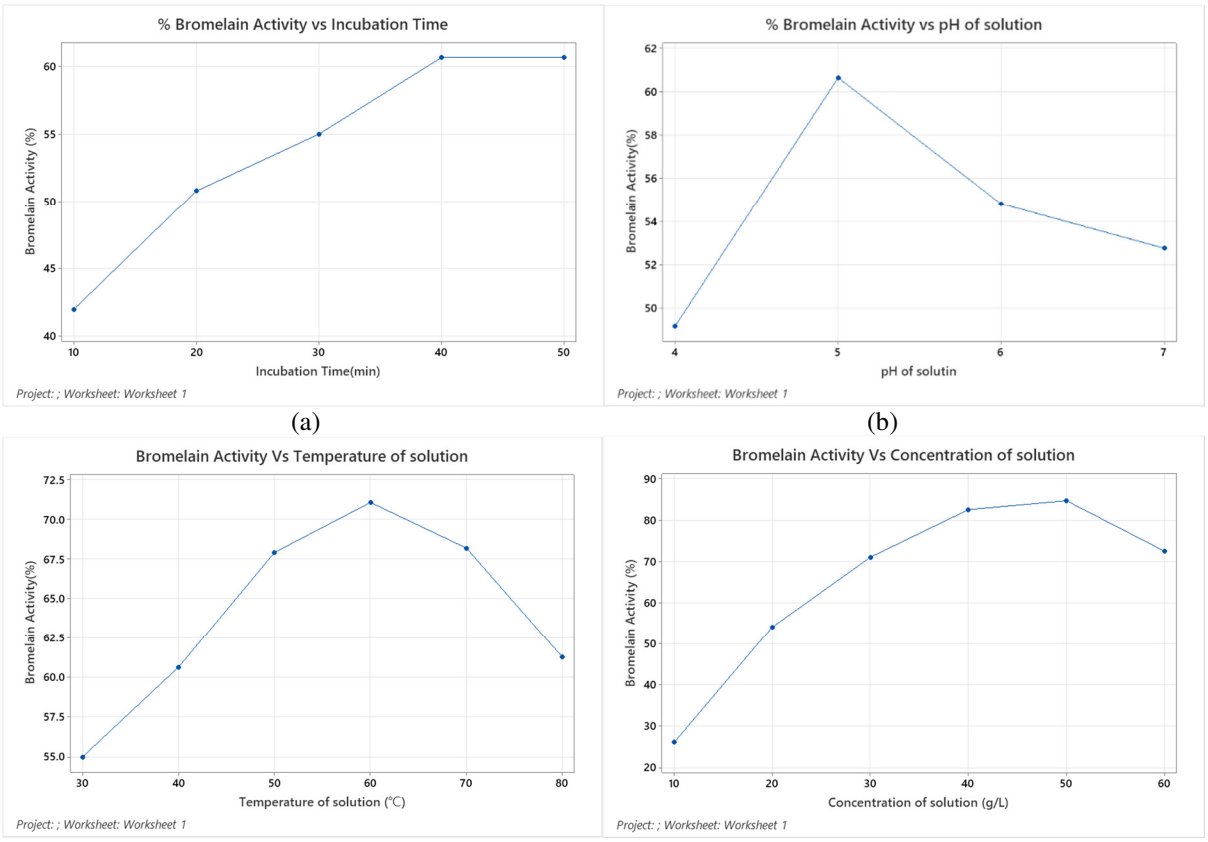


Fig.2. (a – d) Bromelain activity (Papaya) for varying Incubation Time, pH, Temperature and Concentration of Solution

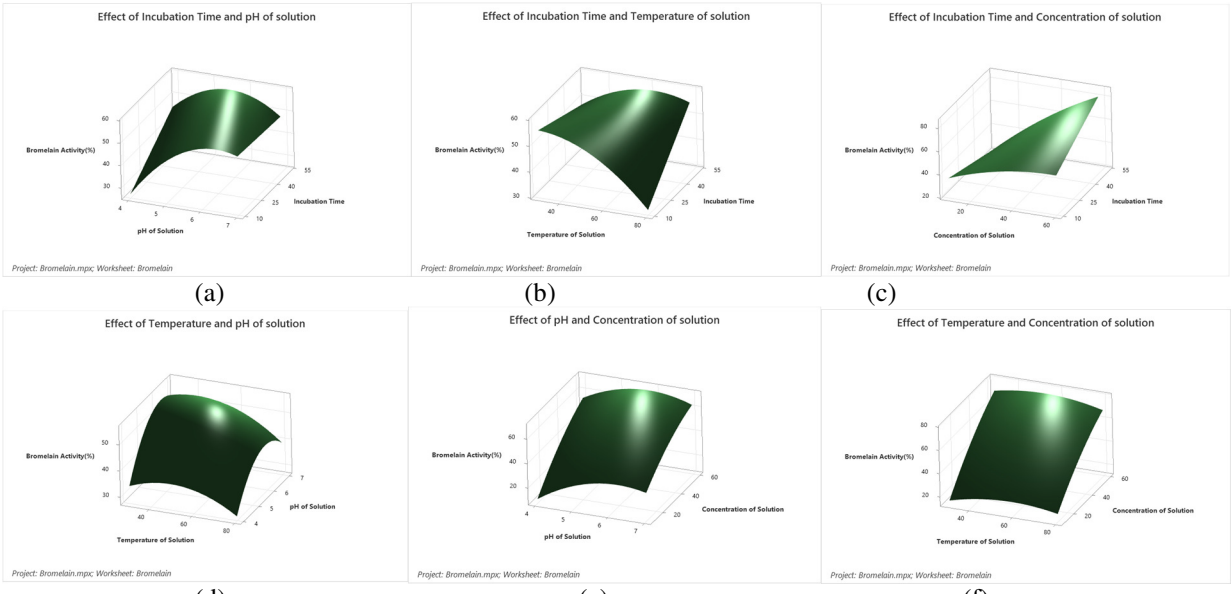


Fig.3.(a-f) Parameters and interactions of X1,X2,X3 and X4(Papaya Source)

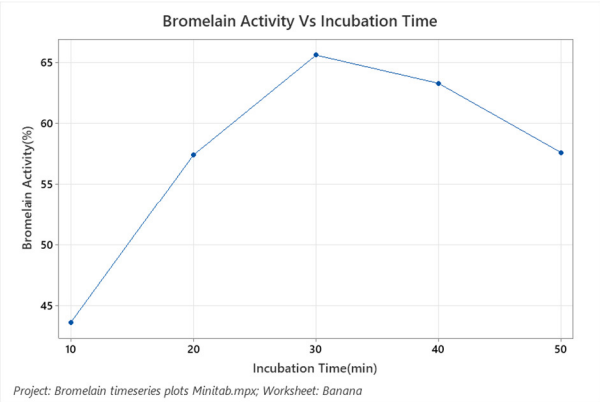
3.2. Bromelain activity from Banana source:

Based on the experiment runs performed, by maintaining pH at 4, concentration at 30g/L, Temperature at 40℃, varying incubation times were analysed using UV Visible Spectrophotometer and optimum Bromelain activity was seen at 30minutes of incubation time beyond which the activity was reduced slightly. At 30minutes of incubation time, at a concentration of 30g/L and Temperature at 40℃, pH of solution was optimized and the optimum value was found to be at 5 where the bromelain activity was more when compared to the other pH levels maintained from 4 to 7. At optimum incubation time and pH, solution maintained at 30g/L concentration, Temperature of solution was varied which was observed to be optimum at 50℃. Maintaining optimum incubation time, pH, Temperature, concentration of solution was varied and optimized at 50g/L which is shown in Fig.4.(a-d). Higher Bromelain activity was observed at Incubation time of 30min, pH of 5, Temperature of solution at 50℃ and concentration of solution maintained at 50g/L.

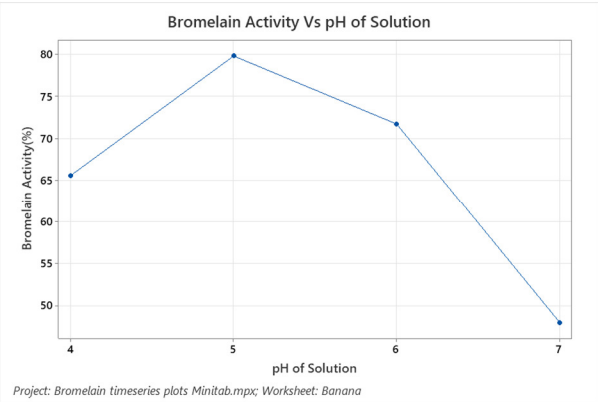
Interactions between the variables and optimum levels for maximum response was shown in Fig.5.(a-f). From RSM using Box Behnken design for optimization of parameters[18,19], the model F-value of 302.73 indicated that the model is significant and P-Value is observed to be <0.05 for model which indicates the parameters and the terms are significant. The "Pred R-Squared" of 0.9838 is reasonable when "Adj R-Squared" is observed to be at 0.9939. Based on the parameters and the significant factors in ANOVA, the coefficients were estimated for the model and the regression equation in coded factors is given as follows:

$$Bromelain\ Activity(Banana\ Source) = 68.85 + 2.32X1 + 2.139X2 - 1.634X3 + 31.158X4 - 12.031X1^2 - 18.555X2^2 - 11.167X3^2 + 2.877X4^2 + 3.669X1X2 + 8.128X1X3 - 1.119X1X4 - 8.688X2X3 + 0.049X2X4 + 2.369X3X4. \quad (2)$$

From the Eq.2, it can be inferred that the increase in incubation time, pH and concentration would ultimately result in optimum bromelain activity, however increasing temperature beyond a certain level would reduce the activity of bromelain. Some interactions and square of these factors have inverse relation with the activity of bromelain. The interdependency among variables like incubation time, temperature of solution and pH are observed to be the prominent parameters for bromelain extraction from RSM and experimental results as well.



(a)



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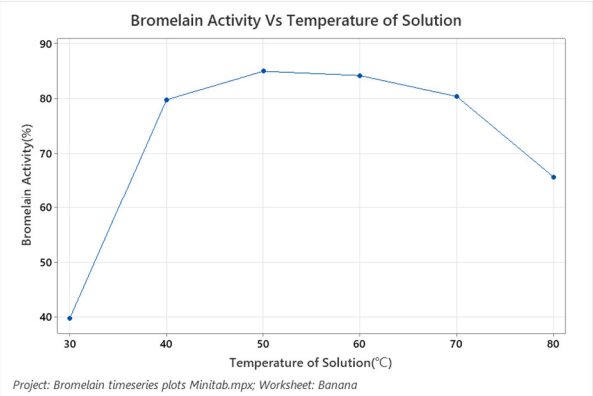

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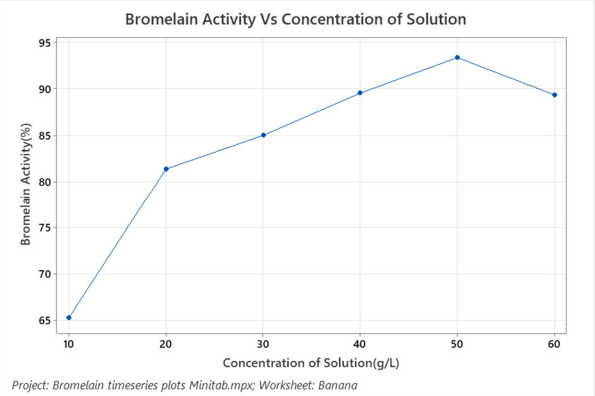


(d)

Fig.4.(a-d) Bromelain activity(Banana) for varying Incubation Time, pH, Temperature and Concentration of Solution

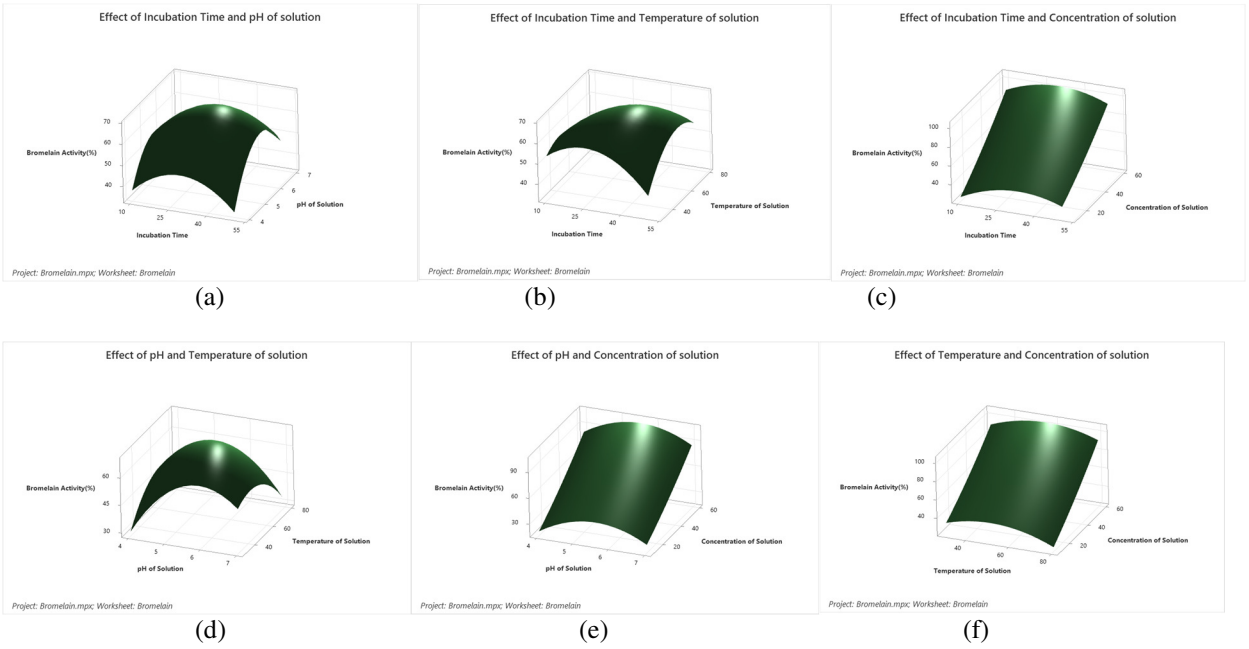


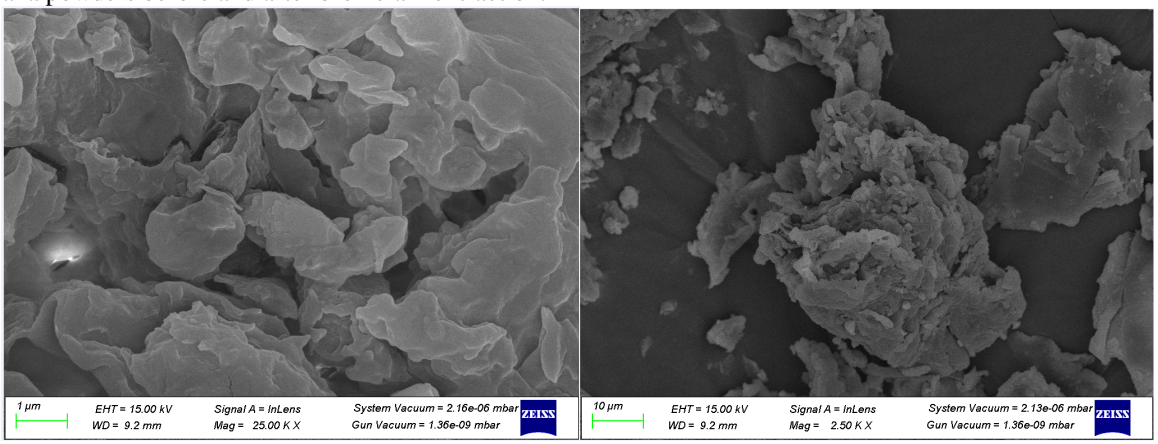
Fig.5.(a-f) Parameters and interactions of X1,X2,X3 and X4(Banana Source)

4. Characterization of Powders:

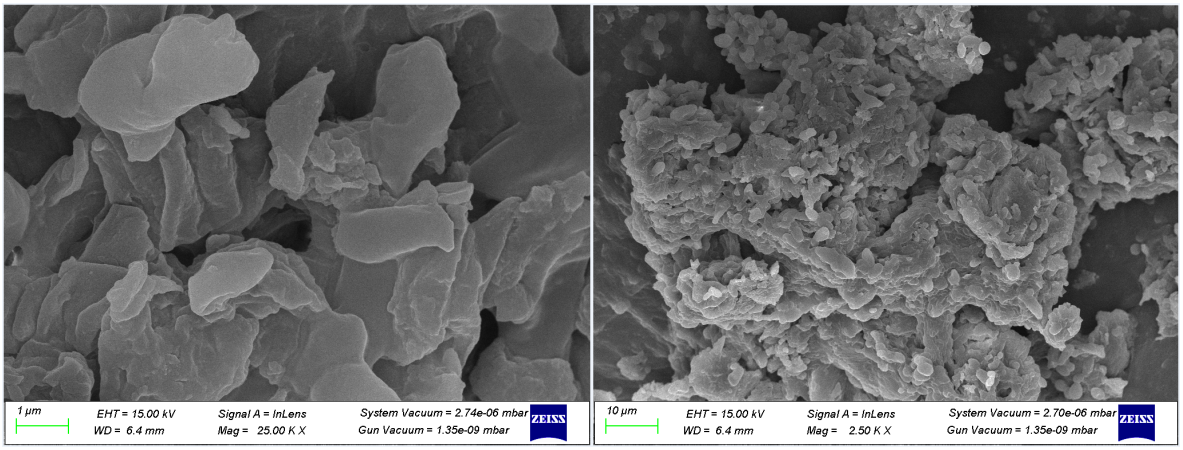
Along with the sources of unripe papaya peel and banana peel waste powders, post recovery of bromelain enzyme from the powders the left-over masses were further dried at 60°C for 2h on a heating mantle and grinded into fine powder. These powdered samples were characterized for SEM, XRD, BET, TGA, DTA and FTIR.

Scanning Electron Microscopy (SEM) was performed to investigate the structural and morphological confirmation of produced nanoparticles. It is obvious that particles carry out the spherical structural creation[15,16,17]. For Papaya, particles have a particle size of between 305nm to several microns whereas for Banana, particle size range from 280nm to several microns. Fig. 6.1(a,b & c,d) indicates the unripe papaya peel waste before and after Bromelain extraction, Fig. 6.2(a,b) indicates the banana peel waste before and after Bromelain extraction. Fig.6.3. indicates the elemental composition of papaya and banana powders before and after the extraction of bromelain enzyme. Post bromelain extraction it was observed that the major constituents include only C, O and N. Source Papaya peel powder contain major portions of C,O and N, traces including K,Br,Mg and P. Banana peel powder have C,O,K in higher portions and the rest including N,Ca,Mg,Fe,Na and P. Banana peel powder is rich in K when compared to papaya peel source.

Papaya and Banana sources were analyzed by XRD, Fig.6.4(a,b & c,d) shows the XRD patterns of Papaya and banana powders before and after bromelain extraction.

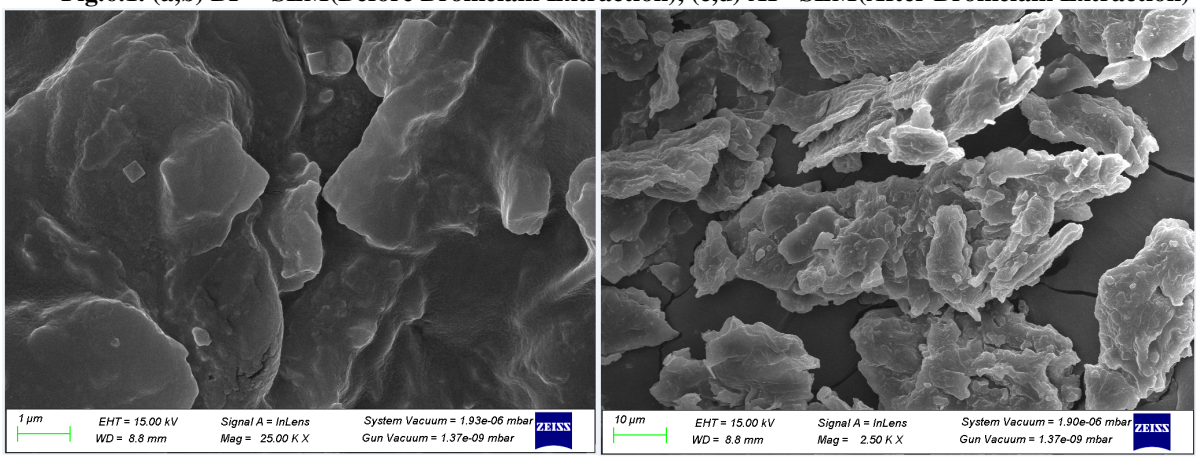


(a) (b)

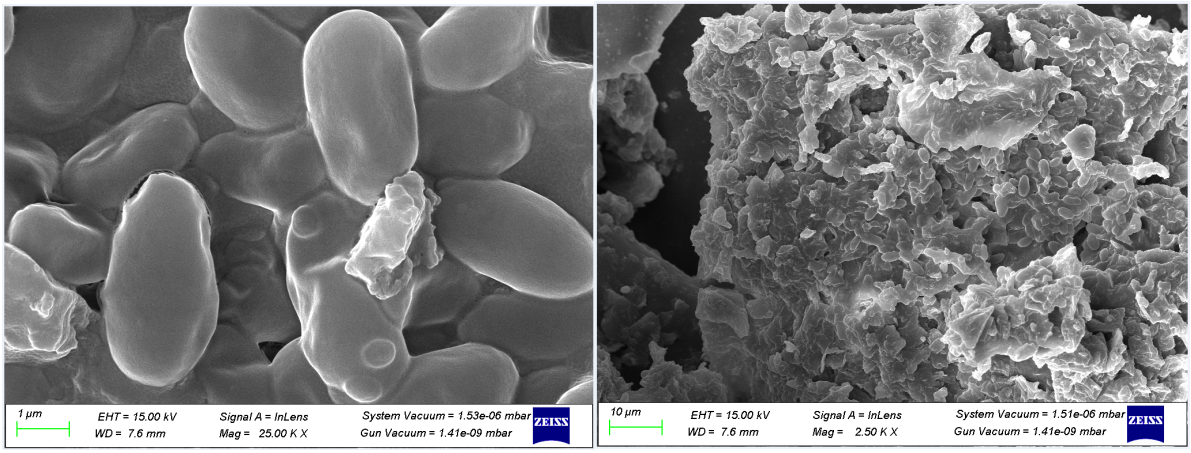


(c) (d)

Fig.6.1. (a,b) BP – SEM(Before Bromelain Extraction), (c,d) AP -SEM(After Bromelain Extraction)

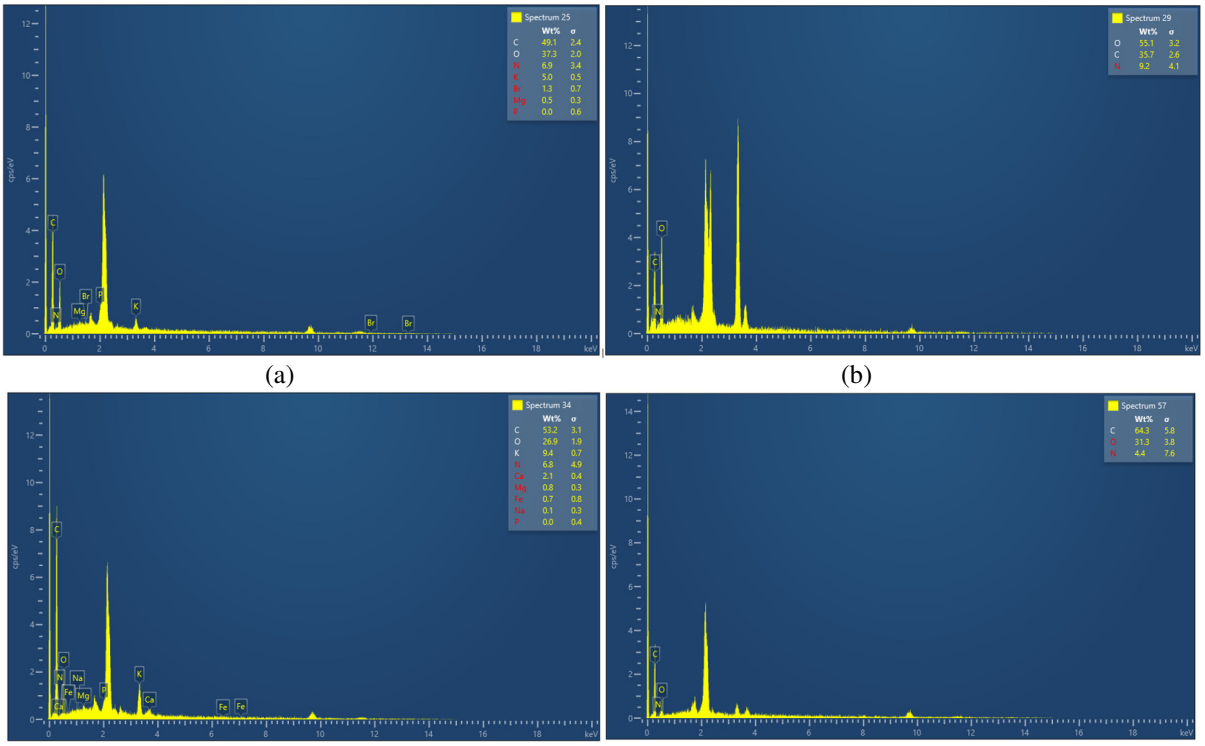


(a) (b)



(c) (d)

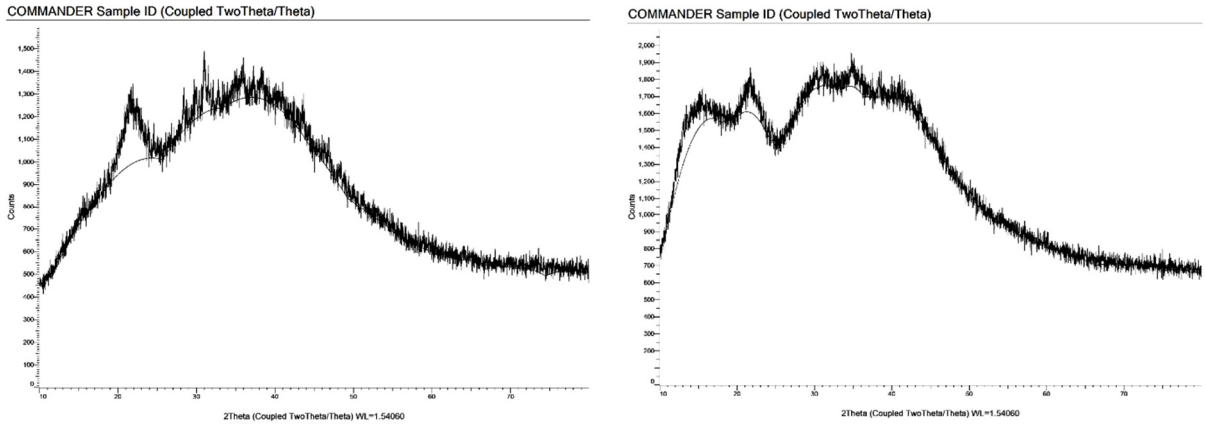
Fig.6.2. (a,b) BB – SEM(Before Bromelain Extraction), (c,d) AB -SEM(After Bromelain Extraction)



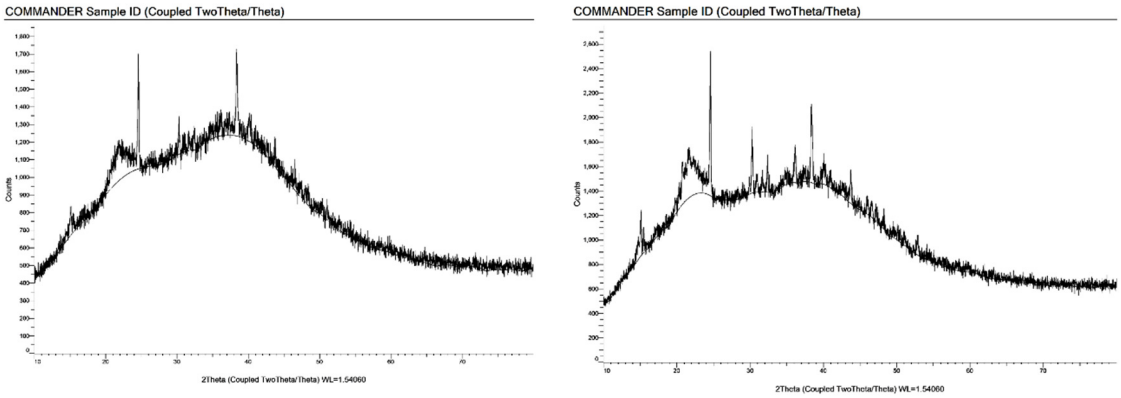
(a) (b)

(c) (d)

Fig.6.3 (a,b & c,d) Elemental composition in Papaya and banana powders before and after Bromelain extraction



(a) (b)

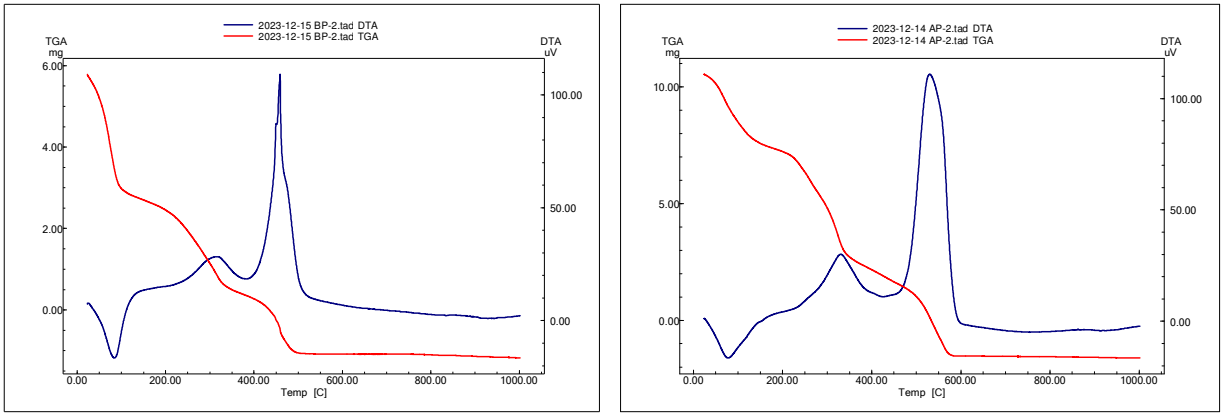


(c) (d)

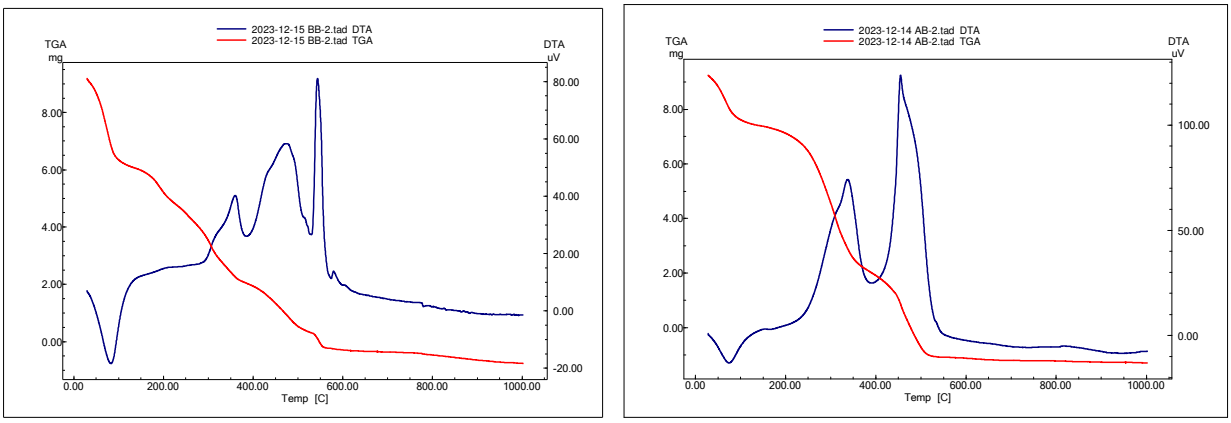
Fig.6.4. XRD pattern of (a,b) Papaya and (c,d) Banana powders before and after the extraction of bromelain enzyme

Brunner Emmett Teller(BET) reveals that the surface area of papaya powder before bromelain extraction is 10.69m^2/g after extraction it is 3.867m^2/g, surface area of banana powder before the extraction of bromelain enzyme is 15.527m^2/g after extraction it is 9.811m^2/g. From which it can be inferred that Banana powder has more surface area before and after bromelain extraction when compared to papaya.

Thermogravimetric Analysis(TGA) & Differential Thermal Analysis(DTA) were performed for both Papaya and banana powders. From Fig. 6.5 it can be inferred that for Papaya samples before bromelain extraction, from TGA weight loss of 119.748% was seen, from DTA, endothermic reaction was seen at 83.99℃ and 382.47℃ followed by exothermic reaction at 458.95℃ releasing 3456.4J/g whereas for samples after bromelain extraction, weight loss of 115.231% was observed, heat was absorbed at 77.89℃ followed by the release of heat at 330.42℃ and 530.03℃ releasing 4070.3J/g. For Banana samples, before bromelain extraction, weight loss of 108.244% was seen from DTA it was revealed that endothermic reaction occurred at 82.9℃ followed by exothermic reactions at 360.11℃, 530.13℃and 580.63℃. For samples after bromelain extraction, weight loss of 114.04%, endothermic reaction occurred at 74.76℃ followed by exothermic reaction at 337.36℃ and 454.86℃ releasing 3908.76J/g.



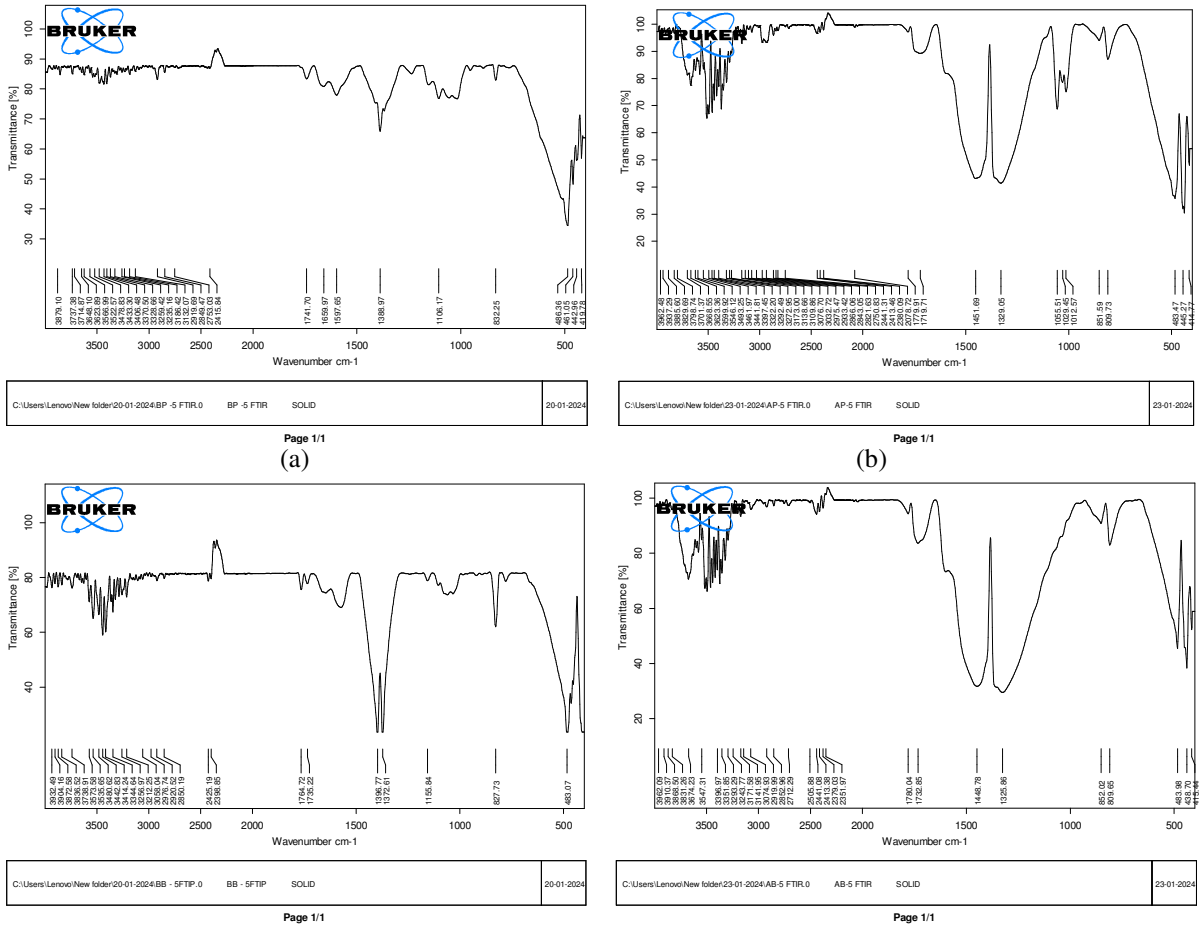
(a) (b)



(c) (d)

Fig. 6.5. (a,b & c,d) TGA & DTA of Papaya and Banana powders before and after bromelain extraction

FTIR spectrum[23] was analysed for the identification of functional groups in Before and after papaya and banana treated powders. Fig. 6.6(a,b and c,d) represents the before and after papaya, banana powder wastes. In papaya powder sample, before bromelain extraction presence of amine, peroxides along with minor presence of aldehydes whereas for the papaya samples after bromelain extraction, presence of peroxides, amines, hydroxides, alkenes. Before bromelain extraction, banana powders include the groups of aldehydes, aliphatic organ halogen compounds, thiols and N-H stretch linkages for the samples post bromelain extraction the presence of aldehydes, N-H groups(primary and secondary amino) and common inorganic ion presence was observed.



(a) (b)

(c) (d)

Fig.6.6. (a) BP – FTIR(Before Bromelain Extraction), (b) AP -FTIR(After Bromelain Extraction), (c) BB – FTIR(Before Bromelain Extraction), (d) AB -FTIR(After Bromelain Extraction)

From GC/MS analysis, for banana bromelain solution, major portion around 13.6% area was occupied by Ethanol, 10.99% 9-Octadecanoic acid (E)-, 9.11% of Dimethylamine, 8.89% of n-Hexadecanoic acid, 8.57% of CO2, 5.92% of cyclohexanol, 4-[(trimethylsilyl)oxy]-, cis-, 4.76% of [1,1'-Bicyclopropyl]-2-octanoic acid, 2'-hexyl-, methyl ester, 4.08% of 1,3,5-Triazine-2,4-diamine, 6-bromo-N,N'-diethyl-, 3.4% of 13-Octadecenal, (Z)-, 2.56% of Octadecanoic acid, 2.46% of 1-Pentanol, 2-[(tert.butoxycarbonyl)amino)- groups and rest being in traces and around and below 1% of the area.

For Bromelain solution obtained from papaya, majority of the groups include 24.05% of carbamic acid, monoammonium salt, 15.97% of 2H-1,2-Oxaborin, 3,6-dihydro-2,3,3-tripropyl-, 14.55% of Propiolic acid, 6.16% of 11,12-Dibromo-tetradecan-1-ol acetate,5.86% of Naphthalene, decahydro-1,1-dimethyl-, 5.68% of Ethanol, 3.61% of 2-Hexanone, 4-hydroxy-5-methyl-3-propyl-, 2.73% of Cyclopentanecarboxylic acid, 2-oxo-, ethyl ester, 2.24% of 2-Formyl-9-[.beta.-d-ribofuranosyl]hypoxanthine and the rest of the groups are present in traces.

5. CONCLUSIONS

Using the unwanted waste materials as resources and by adopting the eco-friendly procedure Bromelain enzyme was synthesized at optimum conditions using unripe Papaya peel waste (Carica Papaya) and banana peel waste (Musa Acuminata).

High Bromelain enzyme activity for gelatin substrate was analysed for optimum conditions. For Bromelain enzyme collected from unripe papaya peel waste source, the optimum activity was seen at a incubation time of 40minutes, pH of 5, Temperature of solution at 60℃ and concentration of solution at 50g/L, the optimum activity observed from papaya source was 84.67%.

Using Banana peel waste as source for bromelain extraction, maximum values were observed to be 93.35% at 30minutes of incubation time, pH of 5, temperature of solution at 50℃ and 50g/L concentration of crude Bromelain solution.

R^2 values obtained from RSM for Papaya and Banana are >95% which shows good fit of the model with the experiment. From the characterizations performed it can be inferred that good nano sized waste powder particles can be seen along with the morphology, surface area, carbon content by weight loss analysis, spectrum, and functional groups.

6. REFERENCES

[1] G. Walsh, “Proteins: Biochemistry and Biotechnology”, London, UK, 1st edition, John Wiley and Sons, 2002.
[2] W. Aehle, “Enzyme in Industry: Production and Application”, 3rd edition, Wiley-VCH Verlag GmbH and Co, Frankfurt, Germany, 2007.
[3] J.Koh, S. -M. Kang, S. -J. Kim, M.-K. Cha, and Y. -J. Kwon, “Effect of pineapple protease on the characteristics of protein fibers”, Fibers and Polymers, 7(2006),180 – 185.
[4] H. R. Maurer, “Bromelain: biochemistry, pharmacology and medical use”, Cellular and Molecular Life Sciences, 58(2001),1234 – 1245.
[5] Muhammed Seid Anbesaw, “Characterization and Potential Application of Bromelain from Pineapple (Ananas comosus) Waste (Peel) in recovery of silver from X-ray films”, International Journal of Biomaterials, Hindawi, 2021, Article ID 9964337, 12 pages.
[6] Resmi Mohan, Venkatasubramanian Sivakumar, Thirumalaisamy Rangasamy and Chellappa Muralidharan, “Optimisation of Bromalain enzyme extraction from Pineapple (Ananas comosus) and application in Process Industry”, American Journal of Biochemistry and Biotechnology, 12(2016),188 – 195.
[7] Mekurialem Demelash Erku, Abubeker Yiman & Anuradha Jabasingh S, “Process Optimization for the recovery of silver from waste X-ray photographic films”, Indian Journal of Chemical Technology, 26(2019),404 – 410.
[8] Addis Engidayehu, Omprakash Sahu, “Enzymatic recovery of silver from waste radiographic film: Optimize with response surface methodology”, Sustainable Chemistry and Pharmacy,15(2020), (100224).
[9] Leticia Celia de Lencastre Novaes, Valeria de Carvalho Santos Ebinuma, Priscila Gava Mazzola, Adalberto Pessoa Junior, “Polymer-based alternative method to extract bromelain from pineapple peel waste”, Biotechnology and Applied Biochemistry, Wiley Online Library, 2013.

[10] H. Umesh Hebbar, B. Sumana, K.S.M.S. Raghavarao, "Use of reverse micellar systems for the extraction and purification of bromelain from pineapple wastes", Bioresource Technology, 99(2008), 4896 – 4902.
[11] Sunantha Ketnawa, Phanuphong Chaiwut and Saroat Rawdkuen, "Aqueous two-phase extraction of bromelain from pineapple peels('Phu Lae' cultv.) and its biochemical properties", Food Sci. Biotechnol., 20(2011),1219 – 1226.
[12] S.S.Gautam, S.K., V. Dash, Amit K. Goyal, G. Rath, "Comparative study of extraction, purification and estimation of bromelain from stem and fruit of pineapple plant", The Thai Journal of Pharmaceutical Sciences, 34(2010).
[13] Sunantha Ketnawa, Phanuphong Chaiwut and Saroat Rawdkuen, "Pineapple wastes: A potential source for bromelain extraction", Food and Bioproducts processing, 90(2012), 385 – 391.
[14] de Lencastre Novaes LC, Jozala AF, Lopes AM, de Carvalho Santos-Ebinuma V, Mazzola PG, Pessoa Junior A., "Stability, purification, and applications of Bromelain: A review", Biotechnology progress, 32(2016),5 – 13.
[15] Bandela Sowjanya,Pulipati King,Meena Vangalapati,and Venkata Ratnam Myneni. "Copper-Doped Zinc Oxide Nanoparticles: Synthesis, Characterization, and Application for Adsorptive Removal of Toxic Azo Dye", international Journal of Chemical Engineering,1(2023)12.
[16] Bandela Sowjanya, Pulipati King, Meena Vangalapati, "Skin of allium sativum (garlic) mediated green synthesis of ZnO nanoparticles and it's adsorption performance for congo red dye removal: kinetic, isotherm and thermodynamic studies", European Chemical Bulletin, 12(2023) 326-332.
[17] U Sirisha, Bandela Sowjanya, H Rehana Anjum, Thanusha Punugoti, Ahmed Mohamed, Meena Vangalapati, "Synthesized TiO2 nanoparticles for the application of photocatalytic degradation of synthetic toxic dye acridine orange", Materials Today: Proceedings, 62(2023)3444-3449.
[18] Basheera Hussain Khatoon, Avapati Surya Lokesh, Lavu Ramadevi, A Ajay Raj, Pulipati King, Meena Vangalapati, "Sodium Lauryl Sulphate removal using copper electrodes without and with perforations by electro coagulation process", Materials Today: Proceedings,59(2022)655-660.
[19] M Subhashita, T Punugoti, B Sowjanya, VR Poiba, "M Vangalapati,Synthesis of Cu/ZnO nanoparticles and its exploitation as a catalyst for the removal of Cetrimonium Bromide", Advances in Materials and Processing Technologies ,8(2022)1880-1888.
[20] D Parveen, VR Poiba, M Vangalapati, "Characterisation and performance of ZnO nano particles for the removal of sodium dodycl sulphate", Advances in Materials and Processing Technologies, 8(2022)913-921.
[21] SI Vali, U Sirisha, VR Poiba, M Vangalapati, P King, "Synthesis and characterization of Titanium doped activated carbon nanoparticles and its application for the removal of dicofol", Materials Today: Proceedings, 44(2021)2290-2295.
[22] C Thummaneni, DVS Prakash, R Golli, M Vangalapati, "Green synthesis of silver nanoparticles and characterization of caffeic acid from Myristica fragrans (Nutmeg) against antibacterial activity",Materials Today: Proceedings, 62(2021)4001-4005.
[23] Asep Bayu Dani Nandiyanto, Rosi Oktiani, Risti Ragadhita, "How to read and inetrpret FTIR Spectroscope of Organic Material", Indonesian Journal of Science and Technology, 4(2019),97-118.