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Optimized Recovery OfSilver From Photographic Waste Using Bromelain Extracted From Musa Acuminata Peel Waste

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ABSTRACT:
Silver prices are rising along with GDP and market. Silver being the most expensive metal was used for many applications, so, it has been quite difficult for the resources of silver to meet its demand. Instead of depleting the resources, research took turn towards search for the possible reuse of available sources. X-rays waste (Used photographic films) was considered as major source for silver recovery in the present research. Recovery was done using Bromelain enzyme extracted from waste Musa Acuminata peel waste. Utilizing the optimized conditions and making use of Bromelain enzyme Silver is recovered in nano scale which has a wide variety of applications in various sectors. Silver recovery was optimized by Response Surface Methodology, experimentation revealed that at a pH of 9, considering 15mL of Bromelain enzyme, by agitating for 40min at 80℃ from 0.6g of X-rays provided optimum silver recovery from photographic films. Characterization was performed from which nano sized silver particles were confirmed from SEM, XRD, surface area was analysed using BET, TGA, FTIR were performed for Silver extracted from Musa Acuminata peel waste.

KEYWORDS: Silver, X-ray film, Bromelain Enzyme, RSM, Musa Acuminata

1. Introduction

Huge motivation for the increased recovery of the silver-like metals was its cost and disposal of radiographic wastes. Declining Silver natural resources resulted in an increased cost of silver. Silver being used for a variety of applications like jewellery, food, medical, electrical, industrial purpose thereby causes burden on the humans causing a peak demand. Source chosen in the current study for the extraction of silver was waste/used x-ray photographic films because the X-ray photographic films once taken during medical examinations will be left unused with time which would further be treated as a radiographic waste and discarded. Instead of stepping towards chemical method of extraction, green synthesis was performed utilizing Bromelain enzyme from waste peels of Musa Acuminata (Banana). Bromelain enzyme hydrolyses the gelatin layer from x-ray sheet for silver extraction. Bromelain enzyme is a natural complex mixture of various enzymes containing cysteine amino acid side chain[1] and it is a combination of different thiol endopeptidases and other constituents like cellulose, glucosidase and other inhibitors[1]. Bromelain is present in fruits, stem and leaves of different plant species; abundant in Pineapple. Muhammed Seid Anbesaw [2] worked extensively on the application of bromelain from pine apple peel in the recovery of silver from waste X-ray films where he utilized the enzyme for around 50

times where he could not observe much loss in the activity of the enzyme. Recovery of Silver from waste radiographic and photographic films was performed by Vinisha[3] et al along with Ag, Zn from zinc ash was also extracted. Hydrometallurgical methods were favourable and NaOH stripping yielded 2.95% and 3.53% by Thiosulphate leaching of Ag by weight and 67.73% of zinc by weight through sulphuric acid leaching. Addis [4] et al utilized bromelain as a biocatalyst in the recovery of silver from waste radiographic film, papain resulted in the recovery of 91.12% of pure silver. Arun[5] et al proposed the removal of silver and polyethylene terephthalate from radiographic films using enzyme protease considering crude enzyme derived from 15 different plant leaves and post smelting 99.8% of silver has been recovered. Jaycee[6] et al optimized the recovery of silver from waste radiographic x-ray films using oxalic acid and shown a recovery of 90.07%. Gilbert U. Tapas kumar[7] et al produced Silver nanoparticles from the recycled produce stable Ag nanoparticles using HPMC(hydroxypropyl methylcellulose), drug delivery was tested. Amira Hassan Al-Abdalall [9] worked on the recovery of silver using protease showing the removal of complete gelatin layer at 50℃, 8 pH and 30min of agitation.JayantP.Parpalliwar[10] et al analysed that with crude protease enzyme within 4 to 6 days complete gelatin layer recovery can be visualized. Adie[11] et al studied on silver extraction potential using NaOH and HNO_3 at varied concentrations and the optimum conditions were found to be 1.5M, 900minutes and 30℃ using NaOH and 4M, 1440minutes and 30℃ with HNO_3, HNO_3 showed higher recovery than NaOH. Utilising Na_2S and NaOH, MekurialemDemelashErku[16] et al recovered silver from waste X-ray film yielding 1.07% at 70.88℃, 10.97 min and 1.05M NaOH concentration. Green route was choosen by Shiv Shankar[17] et al for the synthesis of silver nanoribbons using alkaline protease from which 200 – 400nm nanoribbons were synthesized. Godfrey[18] et al experimented on reduction of $AgNO_3$ by trisodium citrate by in situ X-ray absorption near edge structure spectroscopy and UV-Visible spectroscopy where UV-Vis Spectroscopy was observed to provide better results for Au and Ag. MekurialemDemelashErku[19] et al studied the optimized recovery of silver using NaOH and Na_2S through chemical route of extraction. Shankar[20] et al worked on waste x-ray photographic films from which silver was extracted using alkaline protease from Conidioboluscoronatus, study revealed that Silver in hydrolysate was found to be 3.87 % (w/w) based on total weight of sludge. Rupiasih [22] et al explored the potential of chitosan silver nanoparticle composite in the recovery of silver from waste radiographic films by using the filtration method and high rejection was observed for the Ch-AgNP composite.

Post continued research and after the evaluation of possible sources Bromelain extracted from banana peel waste was taken for the recovery of silver from used x-ray films. The current study mainly focuses on the optimized recovery of Silver from photographic film wastes.

2. Objectives

Using optimized Bromelain enzyme from Musa Acuminata peel waste, recovery of silver was performed by utilizing the design of experiments from Response Surface Methodology. Based on the extraction potential optimum conditions were evaluated for optimum silver recovery. The obtained Silver was further characterised by XRD, SEM, BET, FTIR.

3. Methods

Bromelain Source:

Bromelain enzyme was extracted[2] from local Musa Acuminata peel waste[8] as shown in below fig.1.

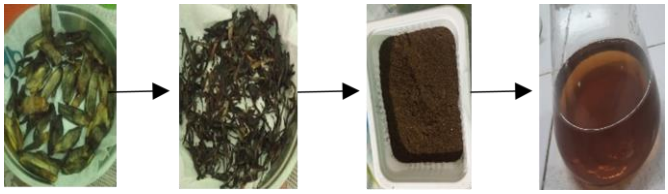


Fig.1. Extraction of Bromelain

Collection of Waste/used X-ray films: X-ray films were collected from a diagnostic centre in Visakhapatnam. Films were thoroughly washed, dried and were cut into pieces of 2cm x 2cm dimension.

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, Borax 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, Muffle Furnace.

Extraction of Crude Bromelain Enzyme:

Powdered sample of Banana was 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℃, the solution was allowed to reach room temperature and then filtered using Whatman filter paper. The filtrate was then 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 Banana peel waste.

Parameters were optimized and optimum conditions of Bromelain @ 50℃, pH of 5, were agitated for 30min, crude bromelain of 50g/L were maintained for the recovery of silver from waste photographic films.

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 $R^2 = 0.9977$.

Extraction of Silver from used X-ray waste:

To extract silver from X-ray waste, optimum conditions were determined as per absorbance and weight loss due to extraction of silver from x-rays.

In all the set of experiments 0.4g of x-ray pieces were added and optimum conditions were determined. At the start of the experimentation, before step 1, temperature of the bromelain solution is maintained at 50℃, pH at 7, 10mL of Bromelain solution was considered for each set with 0.4g of x-ray.

Step 1: Bromelain enzyme activity was determined using 0.4g of x-ray pieces (gelatin layer present in x-rays acts as substrate). 10mL of crude Bromelain solution obtained from banana was considered which was mixed with x-ray pieces and incubated for varying time (Parameter A), at a known pH (Parameter B), known temperature of solution (Parameter C) at known volume (Parameter D) and known weight of x-ray pieces(Parameter E). From 10mL of bromelain solution, 500µL of solution after incubation was considered for the estimation of absorbance from spectrophotometer. After Step 1, weight of x-ray after gelatin layer removal was also noted.
Step 2: After the 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.
Optimization of Parameter A (Step 1) – Effect of Incubation Time:
A 50mL beaker was taken into a digital water bath which is contained with 10mµL of Crude enzyme (from Banana) and 0.4g of x-ray pieces maintained at a pH of 7, concentration of 50g/L at 50℃. 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 banana source.
Optimization of Parameter B (Step 1) – Effect of pH of Solution:
500µL of Bromelain Enzyme from banana mixed with 0.4g of x-ray pieces were taken into a beaker kept in a water bath maintained at a concentration of 50g/L at 50℃ and optimum incubation time of 40 minutes were maintained for the bromelain studies obtained from banana sources. At varying pH like 4,5,6,7,8,9 and 10 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 9 for Banana source.
Optimization of Parameter C (Step 1) – Effect of Temperature of Solution:
500µL of Bromelain Enzyme and 0.4g of x-ray pieces were taken into a beaker which was maintained in a digital water bath at a concentration of 50g/L, optimum pH of 9, and optimum incubation time of 40 minutes were maintained. At varying solution temperatures like 30℃, 40℃, 50℃, 60℃, 70℃, 80℃ and 90℃; samples were collected, and Steps from 2 to 5 were followed. Post analysis in UV Visible Spectrophotometer, optimum solution temperature was found to be 80℃ for Banana Bromelain source.
Optimization of Parameter D (Step 1) – Effect of Volume of Solution:
Bromelain solutions of 5mL, 10mL, 15mL, 20mL and 25mL were considered. Varying volumes of Crude enzyme (Banana Bromelain Enzyme) at 50g/L was considered and 0.4g of x-ray pieces were taken into a beaker which was maintained in a digital water bath at optimum temperatures of bromelain from banana maintained at 80℃, optimum pH of 9, and optimum incubation time of Bromelain enzyme obtained from Banana(40minutes) were maintained. At varying volumes, samples were collected, and Steps from 2 to 5 were followed. Post analysis in UV Visible Spectrophotometer, optimum volume of solution was found to be 15mL for Banana Bromelain sources.

Optimization of Parameter E (Step 1) – Effect of weight of x-ray pieces:
Considering optimum volume of 15mL Bromelain solutions, at 50g/L was considered and varying weights of 0.2g, 0.4g, 0.6g, 0.8g and 1.0g of x-ray pieces were analysed in the study. At varying weights, incubation was performed in a digital water bath at bromelain from banana maintained at 80℃, optimum pH of 9, and optimum incubation time of Bromelain enzyme obtained from Banana(40minutes) were maintained. At varying weights, samples were collected, and Steps from 2 to 5 were followed. Post analysis in UV Visible Spectrophotometer, optimum weight of x-ray was found to be 0.6g for Banana source.
Silver Extraction:
At these optimum conditions, the extracted silver layer was further dried in muffle furnace and equal quantities of Borax and Na_2CO_3 are added to get pure silver.

Fig.2. Pure Silver post drying in Muffle Furnace

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 Volume of solution(X4, mL) and weight of x-rays(X5, g). In total 46 experimental runs were performed by considering all the 5 factors X1, X2, X3, X4 and X5 at Low, Medium and High levels. Table.1. represents the range and levels using for optimization of Bromelain activity from banana peel sources. Minitab 20.2 (64-bit) statistical software was used for the analysis. Fig.3, and Fig.4. indicate the model summary and Analysis of Variance for Bromelain activity of Banana source. Table.1 represents the parameters and the range considered for the optimization, Fig 4 represent the DoE and the interactions among parameters.

Table.1. Parameters, Range and Levels (Bromelain enzyme obtained from Banana Source for Silver removal from x-rays)

Parameters	Range	Low (-1)	Medium (0)	High (+1)
Incubation Time, min	10 – 50min	10	30	50
pH of solution	4 – 10	4	7	10
Temperature of solution, ℃	30℃ – 90℃	30	60	90
Volume of solution, mL	5mL – 25mL	5	15	25
Weight of x-rays, g	0.2 – 1.0 g	0.2	0.6	1.0

As per the analysis of independent and dependent variables, quadratic equation was obtained for the activity of Silver from banana source.

4. Results

Bromelain activity for silver removal:

By experimentation, utilizing the bromelain at optimum conditions, absorbance was noted down 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 volume of bromelain solution at 15mL and Temperature at 80℃, pH of solution was optimized and the optimum value was found to be at 9 where the bromelain activity was more. At optimum incubation time and pH, solution maintained at 50g/L concentration, Temperature of solution was varied for which the optimum temperature is seen at 80℃. Maintaining optimum incubation time, pH, Temperature, volume of solution was varied and optimized at 15mL, weight of x-rays at 0.6g which is shown in Fig.3.(a-e) Higher Bromelain activity was observed at Incubation time of 40min, pH of 9, Temperature of solution at 80℃ and volume of solution at 15mL and 0.6g of x-ray pieces.

Interactions between the variables and optimum levels for maximum response was shown in Fig.4.(a-j). From RSM using Box Behnken design for optimization of parameters, the model F-value of 136.37 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 0.9689 is reasonable when "Adj R-Squared" is observed to be at 0.9837. 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 (X1 - Time, X2-pH, X3-Volume, X4-Temperature, X5-Weight of x-rays).

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
1.48122	99.09%	98.37%	96.89%

Fig.3. Model Summary of BBD

Analysis of Variance

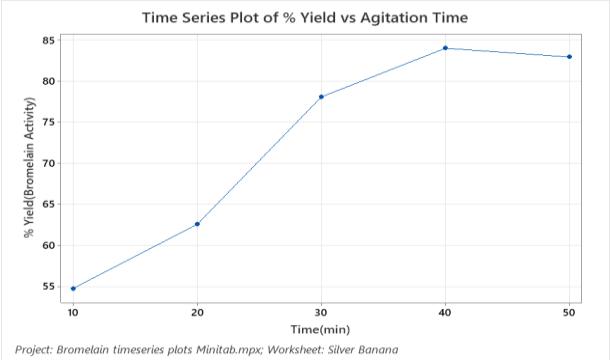
Source	DF	Adj SS	Adj MS	F-Value	P-Value
Model	20	5984.08	299.20	136.37	0.000
Linear	5	4707.03	941.41	429.08	0.000
Agitation Time	1	1156.26	1156.26	527.01	0.000
pH of Solution	1	2907.99	2907.99	1325.43	0.000
Volume of Solution	1	2.36	2.36	1.07	0.310
Temperature of Solution	1	387.94	387.94	176.82	0.000
Weight of X-rays	1	252.49	252.49	115.08	0.000
Square	5	745.86	149.17	67.99	0.000
Agitation Time*Agitation Time	1	58.63	58.63	26.72	0.000
pH of Solution*pH of Solution	1	687.02	687.02	313.14	0.000
Volume of Solution*Volume of Solution	1	203.36	203.36	92.69	0.000
Temperature of Solution*Temperature of Solution	1	61.21	61.21	27.90	0.000
Weight of X-rays*Weight of X-rays	1	96.83	96.83	44.13	0.000
2-Way Interaction	10	531.18	53.12	24.21	0.000
Agitation Time*pH of Solution	1	32.59	32.59	14.86	0.001
Agitation Time*Volume of Solution	1	12.50	12.50	5.70	0.025
Agitation Time*Temperature of Solution	1	12.56	12.56	5.72	0.025
Agitation Time*Weight of X-rays	1	9.45	9.45	4.31	0.048
pH of Solution*Volume of Solution	1	4.30	4.30	1.96	0.174
pH of Solution*Temperature of Solution	1	142.95	142.95	65.15	0.000
pH of Solution*Weight of X-rays	1	65.86	65.86	30.02	0.000
Volume of Solution*Temperature of Solution	1	137.78	137.78	62.80	0.000
Volume of Solution*Weight of X-rays	1	18.53	18.53	8.45	0.008
Temperature of Solution*Weight of X-rays	1	94.67	94.67	43.15	0.000
Error	25	54.85	2.19		
Lack-of-Fit	20	42.41	2.12	0.85	0.643
Pure Error	5	12.44	2.49		
Total	45	6038.93			

Fig.4. ANOVA of Optimization of Recovery of Silver

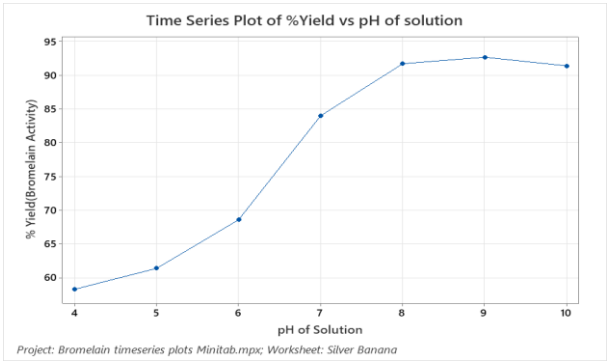
Note: Bromelain activity and yield are used interchangeable.

$$Bromelain\ Activity(Banana\ Source) = 82.667 + 8.501X1 + 13.481X2 + 0.384X3 + 4.924X4 + 3.972X5 - 2.592X1^2 - 8.873X2^2 - 4.827X3^2 - 2.648X4^2 - 3.331X5^2 + 2.855X1X2 - 1.768X1X3 + 1.772X1X4 - 1.537X1X5 + 1.036\,X2X3 + 5.978X2X4 + 4.058X2X5 + 5.869\,X3X4 + 2.152\,X3X5 + 4.865\,X4X5. \quad (2)$$

From Eq.2. as positive and negative coefficients were found for some interactions, positive coefficients maximize the activity whereas negative coefficients minimizes the activity of bromelain/yield. We can infer that the increase in incubation time, pH, volume, Temperature and weight of x-rays would ultimately result in optimum bromelain activity. Some interactions and square of these factors have inverse relation with the activity of bromelain. The effect of volume of solution and weight of x-rays with agitation time shows inverse relation indicating the prominence of interaction of variables on the recovery of silver by RSM. The experimental results were in close correlation with the optimum conditions obtained from RSM.



(a)



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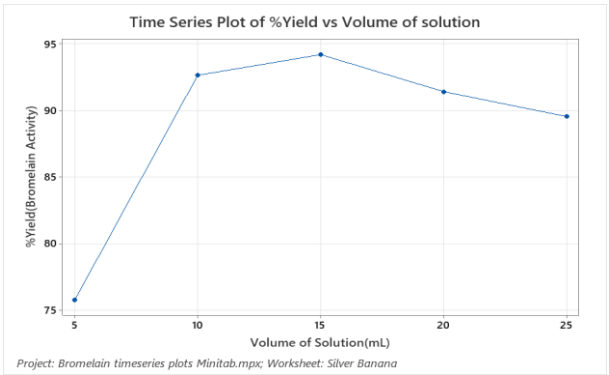

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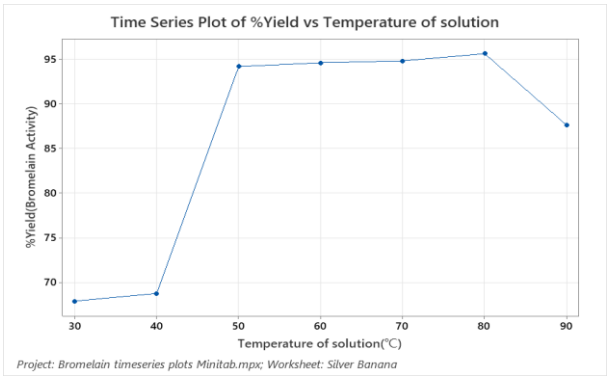

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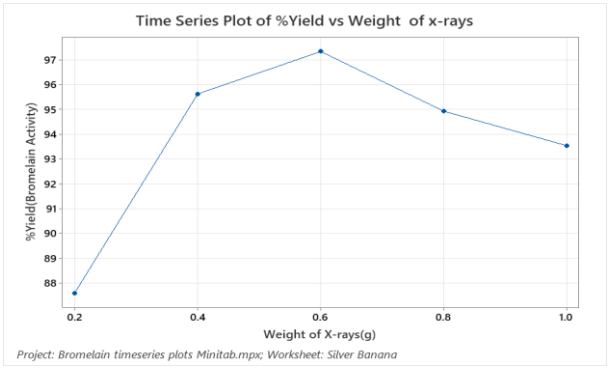
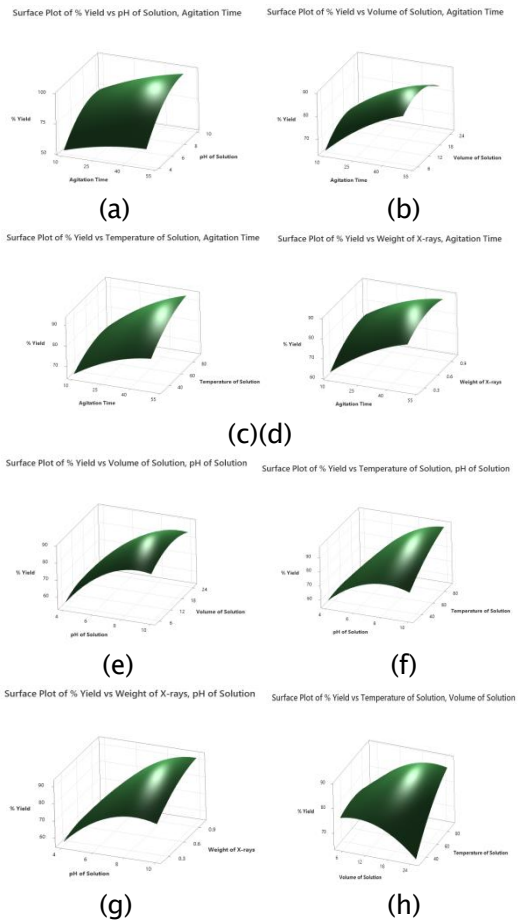
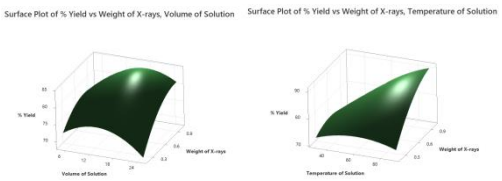


(e)

Fig.3. (a – e) Bromelain activity (Banana) for varying Incubation Time, pH, Temperature, Volume of Solution and weight of x-rays for the removal of silver





(i) (j)

Fig.4.(a-j) Parameters and interactions of X1,X2,X3, X4 and X5(Silver Extraction from Banana Source)

Characterization of Powders:

Prior to the treatment in muffle furnace at 950℃, the hydrolysed gelatin layers were further dried in a furnace at 500℃ for 30min. The dried powders were grinded into fine powder. These powdered samples were characterized further.

X-ray diffraction patterns of Fig.5. indicates the presence of Silver in the gelatin layer extracted from x-rays using bromelain solution extracted from banana peel source. High intense peaks were observed at [12] 2θ = 38.3°, 44.5°, 64.6° and 76.8° respectively thereby representing the silver crystals at a plane of (111), (200), (220) and (311). This XRD pattern indicates that the extracted silver nanoparticles are face-centered, cubic and crystalline in nature. At 32.3° a peak was observed indicating the presence of oxides of silver.

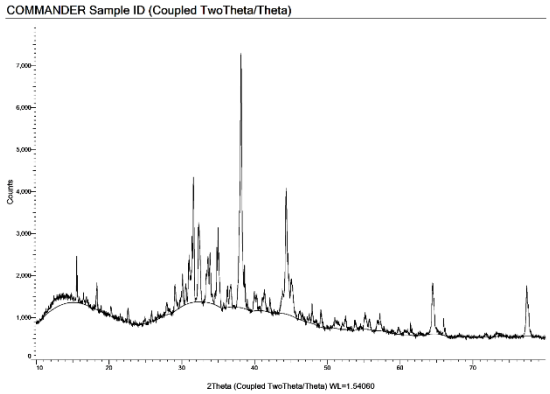
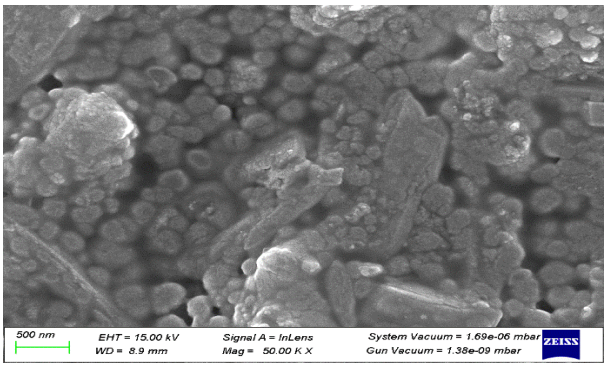
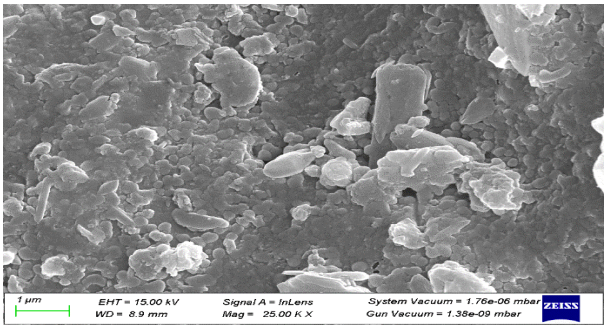


Fig.5. XRD of gelatin layer extracted from X-rays using bromelain from banana peel source

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[14,15,16]. For silver extracted from gelatin layers of x-ray waste sized from 20nm to 300nm. Fig.6. (a,b) indicates the silver nano particles extracted from gelatin layers of x-ray waste.



(a)



(b)

Fig.6. a,b. Silver nano structures in extracted gelatin layer.

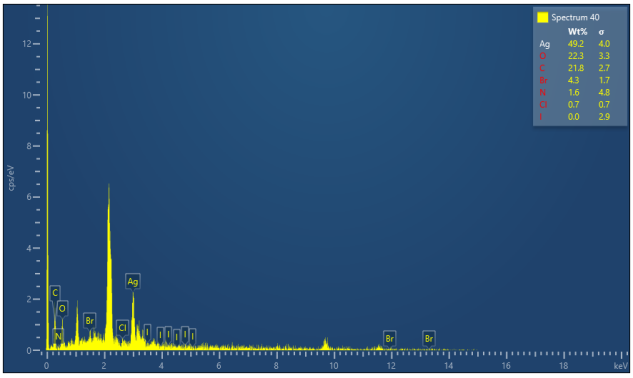


Fig.7. Elemental analysis in extracted gelatin layer

The above figure 7 indicates the elemental composition in resources and product. Where the presence of Silver in higher amounts followed by elements like O,C,Br,N,Cl,I.

Thermogravimetric Analysis (TGA) & Differential Thermal Analysis (DTA) were performed for silver powder. In Fig. 8. for the silver obtained from gelatin layer, endothermic reaction occurred at 77.81℃ and 109.8℃ followed by exothermic heat release at 519.96℃ with a heat release of 650J/g. From TGA, weight loss of 3.684mg (43.972%) was observed for gelatin layer powder.

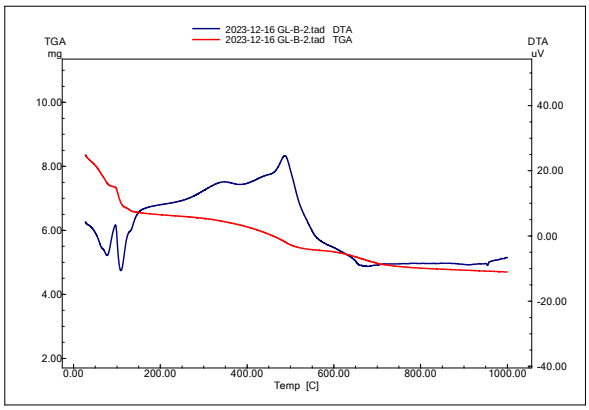


Fig.8. DTA & TGA of gelatin layer

Brunner Emmett Teller (BET) [17] analysis reveals the surface area for obtained Silver of 10.998 m^2/g from gelatin layer with a pore volume of 0.039 cc/g and a diameter of 3.13nm. Fig.9. reveals a good adsorption-desorption characteristics of silver.

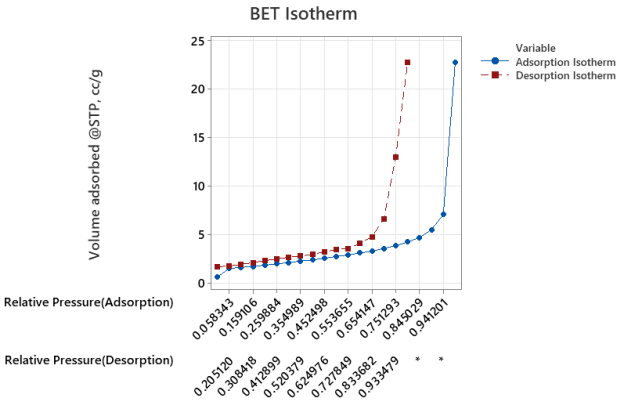


Fig. 9. BET – Adsorption-Desorption Isotherm of Silver

Fourier Transform Infrared Spectroscopy (FTIR) analysis concluded[21] the presence of hydrate, hydroxyl, ammonium or amino due to the presence of broad absorption band between 3650 and 3250 cm^{-1}, peak in the range of 2700 and 2800 cm^{-1} represents the existence of aldehydes, the existence of peaks below 1700cm^{-1} represents the amides or carboxylates functional groups. Fig.10 shows FTIR spectra for the gelatin layer post heat treatment in muffle furnace.

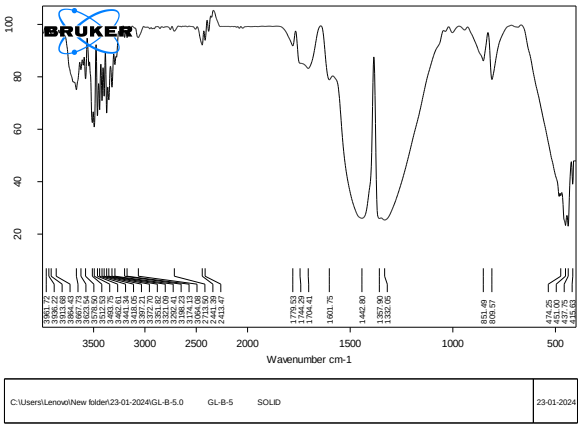


Fig.10. FTIR – Gelatin Layer obtained waste x-ray films using bromelain from banana peel waste.

5. Discussion

Utilizing the least utilized waste materials as resources like banana peels and by adopting the eco-friendly procedure Bromelain enzyme was synthesized at optimum conditions using Musa Acuminata waste.

At optimum operating conditions Bromelain was utilized for the recovery of silver from waste/used X-ray photographic films. Recovery of silver from Bromelain was optimised at 80℃, pH of 9, using 15mL of Bromelain solution with 0.6g of x-ray pieces and agitating the solution for 40minutes provided the recovery of 97.33%.

The process was optimized using RSM. R^2 values obtained from RSM for Silver extraction was observed to be under acceptable range indicating good fit of the model with the experiment.

From the characterization techniques performed it can be inferred that good nano sized silver particles were observed along with the morphology, spectrum, and surface area.

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