Extraction of Keratin from Human Hair with Production of Biofertilizer from Waste Liquid of Hair Extraction and its Efficient Application on Growth Yield of *Abelmoschus esculentus* L.

Gayathri Unnikrishnan[1],*, Vijayaraghavan Ramasamy[2]

[1]Department of Microbiology, Nehru Arts and Science College, Coimbatore, Tamil Nadu, INDIA.
[2]Dean-Bioscience, Nehru Arts and Science College, Coimbatore, Tamil Nadu, INDIA.

ABSTRACT

Keratin has been attracting interest due to its stability against enzymatic degradation thereby allowing more predictable degradation profile for tissue regeneration. While the efficacy of keratin has been demonstrated in different tissue models, there has been no systematic study to investigate and compare the different routes of keratin extraction from human hair. Here, we introduce a technique for mass keratin extraction from human hair. Keratin was extracted from human hair using 7 M urea, 6g SDS and 15 ml of 2- mercaptoethanol. It resulted in 73 % yield of about 6.8 g of keratin from 10 g of human hair waste and low crystallinity but the protein formed aggregates with highest hydrodynamic average size of around 5 μm. However, to study the fertilizing property of waste water generated from keratin extraction, they are applied on *Abelmoschus esculenthus* L. and recorded its biometric characteristics for 90 days. It is observed that nitrogen loss is the drawback for hair waste fertilizer, meanwhile in this study, we have observed minimal nitrogen loss of <15 % from this hair waste water application. These results provide new insight into the extraction of keratin from human hair with implications for its use as an organic fertilizer.
Key words: Keratin, Hair waste, Fertilizer, FE-SEM, Okra.

Correspondence:
Dr. Gayathri Unnikrishnan,
Scholar, Department of Microbiology, Nehru Arts and Science College, Coimbatore, Tamil Nadu-641105, INDIA.
Phone no: +91 7418820568

Email: gay3avittam2012@gmail.com

INTRODUCTION

Keratins are abundantly available as by-products mainly from slaughterhouses and poultry plants in the form of skin wastes, hair, horns, hooves, feathers and claws etc., which have been wasted without adequate utility. As per the European Parliament and Council regulation EC 1774/2002, keratin waste has been classified as category 3 material, stating that it is not intended for human consumption; however, the same does not transmit diseases to humans and animals.[1] Limits imposed on the processing of animal by-products for feedstuffs and the subsequent increase in the unused amount of keratin waste have enhanced an interest in methods of their natural and agricultural management. Attempts to use waste feathers in the production of fertilizing agents, such as keratin-bark-urea granulates, were made in Poland as early as in the 1980s.[2,3] Microplot experiments showed that such fertilizing agents favourably influenced soil properties and some crop plants,[2-5] at the same time limiting patch weeding.[6] However, their influence on the microbiological activity and nitrogen transformation in the soil was not uniformly positive. A study in previous showed that the fertilizer caused a reduction in the respiratory activity of light soils, disturbances in the nitrification process and nitrogen losses from this environment.[7] Traditionally, keratin has been extracted from animal hard tissues including wool, feathers, hooves and horns especially for use in the cosmetic industry.[8-10] However, keratin extraction from animal tissue requires harsh conditions and may induce xenogeneic response, limiting its applicability

in biomedical research. To circumvent the limitations with xenogeneicity, human hair was used as substrates to extract keratin. Different methodologies of keratin extraction from human hair have been developed over the years predominantly focusing on the cleavage of disulfide linkages on keratin protein backbone in order to enhance its aqueous solubility. However, each of these methods have been studied in isolation and are neither fully characterized nor compared. It is important to outline the differences in the extracted keratin mainly to streamline its translation into clinical biomaterial and also to compare the biological response observed using keratin obtained from different methods. The main component of human hair is keratotic protein, which is 65–95% of the total mass.[11] A great amount of waste human hair is discarded each year, which causes an environmentally difficult disposal problem. Therefore, from the view of economy and environment protection, it is quite desirable to develop effective and profitable process to use these resources. Currently, many natural proteins have been applied in many functional applications. Films based on human hair keratin were used as substrates for cell culture and tissue engineering.[12] Film made of human hair as a nail plate model was studied drug permeation. The biocompatible and porous keratin-based hydrogels were prepared using electron beam irradiation.[13] However, one of the most serious problems is the extraction of proteins. A large quantity of reagents such as acids or reductants is consumed and cannot be recycled, whereas the costs of ionic liquids are obviously higher than that of inorganic reagents. Thus, researchers have focused on finding simple and ecofriendly processing methods to extracting protein. In this paper, we describe a convenient extraction procedure of proteins containing keratins from human hair wasters by alkali method. Deterioration of soil quality induced by soil salinisation and heavy metal contamination in combination is an ever growing global problem due to climate change and rapid and the rapid development of irrigated agriculture, posing a threat to both environmental and agricultural sustainability in both developing and non-developing countries.[14] Hence in this study we apply the waste water generated after keratin extraction from human hair waste to Okra (*Abelmoschus esculentus* L.) to analyze its fertilizing efficiency.

MATERIALS AND METHODS

Pulverized raw human hair mass were obtained from the local barber shops at Thirumalayampalayam, Coimbatore (India). This material was washed thrice with distilled water to remove all dirt and then drained. After drying it completely in an oven at 100ºC for 2 h, it was used as raw material for further studies. 27 Urea, SDS, 2-mercaptoethanol and dialysis membrane (with 12-14 kDa molecular weight cut off) were purchased from HiMedia (HiMedia Laboratories Pvt. Ltd., India). Hexane was obtained from SDFCL (SD FineChem Ltd., India) while DCM from Merk (Merk Millipore Corporation Ltd., Germany). For characterization techniques; KBr and DMEM were purchased from Sigma-Aldrich (Sigma Aldrich Chemicals Pvt. Ltd., India), FBS from Gibco® (Invitrogen, USA) and coomassie brilliant blue R-250 stain from SRL (Sisco Research Laboratories Pvt. Ltd., India).

Extraction of Keratin from Human Hair

The first step of extraction is defatting i.e. removal of fats from the raw material. Soxhlet's apparatus was used to carry out defatting/delipidization of pulverized hoof sample for two days. Mixture of hexane and dichloromethane (1:1, v/v) was used for refluxing. Ten gram of defatted raw material was mixed with 7 M urea, 6g SDS and 15 ml of 2- mercaptoethanol in a 1000 ml round-bottom flask and kept in an orbital shaker at 60qC for 12 h to extract keratin at pH 7.[15] The resulting solution was then centrifuged for 15 min at 6000 rpm and the supernatant was dialyzed against degassed water for 5-6 days. Some of the extracted keratin was kept in a deep freezer at -80qC for 5 h and lyophilized to make it into powder. Hereafter, the dialyzed keratin, i.e. the one prior to lyophilization, will be mentioned as liquid keratin and the lyophilized one will be mentioned as powder keratin.

Quantification of Protein

The protein content was determined through CHNS analysis. Carbon, hydrogen, nitrogen and sulfur contents of the lyophilized keratin 28 sample were analyzed using varios MICRO V2.0.3 CHNSO. Protein content of the sample was quantified by CHNS analysis based on the amino acids present in hoof keratin. To determine the molecular weight, the liquid keratin was denatured before subjecting it to one-dimensional slab SDS-PAGE (10% gel) by heating the sample mixed with an equal amount of sample buffer containing SDS and beta mercaptoethanol. The concentration of liquid keratin used was about 20 μg per well. The gel was stained using coomassie brilliant blue stain.[16]

Field Emission Scanning electron microscope (FE-SEM)

The surface morphology of sample was observed by FE SEM (Quanta, FEG 250, Japan) and X-Ray Diffraction.

The samples were adhered directly onto a cupreous stage and then sputter coated with athin layer of gold. Digital photographs were captured with 10,000 times magnification at 10 kV and stored in TIFF format.[17,18]

Degraded hair waste water as biofertilizer Plantation and dose application

The okra seedlings (*Abelmoschus esculentus*) were obtained from Central Nursery, Kerala Agricultural University, Thrissur, Kerala, India. Seedling packets purchased were packed on same date and weighed 4 g each. The potting mixture was prepared in (20x10) cm polybags. The seedlings after germination in C-tray were transplanted to poly bags. Plantation was done on 4 November 2019 in the selected plot with spacing of 3x3 cm between two plants and rows consisting15 plantlets with 3 for each treatment. Each set was amended with FDP (Fixed Dose Procedure) as 20 % root dose (RD) and 5 % shoot dose (SD) through fertigation and foliar spray at the interval of 2 days. The control plants without treatment were maintained. All the plants were irrigated regularly through drip irrigation and the experiment was carried out in triplicate. Regular practices required for the cultivation like weeding and earthing up were done routinely.[19]

Physiochemical Composition of Human Hair Waste Water

Quality of waste water generated after each extraction was analyzed for nutrient status using the standard procedure given in Table 1.

Biometric Observations of Seedlings

Biometric observations were recorded biweekly interval for three months. Plant height, number of leaves per plant, plant girth was recorded. Vigour index was also calculated from the biometric observations.

Statistical Analysis

Data were subjected to analysis pf varience (ANOVA)[20] using statistical package 'MSTAT-C' package. Wherever the F test was significant (at 5 % level) multiple comparison among the treatments were done with Duncan's Multiple Range test (DMRT).

RESULTS

Extraction of Keratin from Human Hair

In order to react completely to extract keratin effectively, an excess of 2-mercaptoethanol was used to cleave the disulfide bonds. The optimum pH range adopted for extraction was 6-8, as keratin could not be extracted at acidic pH since 2-mercaptoethanol could not be ionized at low pH values, resulting into loss of reactivity with keratin.

However, at alkaline pH, keratin is likely to undergo decomposition, in turn leading to a low yield of the extracted keratin. Due to the presence of hydrophobic amino acids in keratin, 2-mercaptoethanol could Defatting of humn hair at (1:1 v/v hexane and DCM). Reduction using urea, SDS and 2-mercaptoethanol at 60ºC for 14 h Centrifugation at 6000 rpm for 15 min to collect supernatant. Dialysis of supernatant against water for 5-6 days was followed by lyophilisation.

Quantitative Estimation of Keratin

The lyophilized keratin sample contained 13.3% of nitrogen, 45.3% of carbon and 6.84 % of hydrogen. High amount of hydrogen in our sample does not rule out the possibility of the presence of bound water as one of the constituent of the sample. Hence the purity of the keratin sample is observed to be greater than 80%. Based on the initial weight of the hooves, 44 % yield of keratin was obtained. The molecular weight of the keratin had been estimated by SDS-PAGE using 10% polyacrylamide gel, which showed two clear protein fractions[21] with equal intensities between 45-50 and 55-60 kDa as shown in Figure 1.

Field Emission Scanning Electron Microscope (FE-SEM)

The effects of the protein extraction techniques on the overall morphology of human hair samples were observed by field emission scanning electron microscope (FE-SEM). Compared to normal hair without protein extraction (Figure 2), extracted hairs using SDS, β mercaptoethanol and urea extraction showed significant shrinking and depletion of mass (Figure 4). Based on the observed morphological changes i.e. decrease in thickness, the degree of shrinking, mass depletion, overall damage of the hair surface, number of proteins identified and their abundance, the SDS-Urea extraction techniques were more efficient.

EDAX Spectrum

In the study of energy dispersive X-ray analysis of the human hair before and after keratin extraction, Figure 3 and Figure 5 reports the data on different elements present in male human hair by employing energy dispersive x-ray analysis. Of all elements analyzed C and O in general, are found to be more in quantity before extraction. The percentage of carbon is more i.e., 56% to 43% when compared to other elements. The Energy Dispersive X-Ray Analysis of Human Hair, interesting feature is that, the carbon isotopes of human hairs hold dietary information related to both food sources

and dietary practices in a region. Oxygen is the second highest quantity present in human hair, when compared with the other elements. The hair waste water after keratin extraction shows N, O and Na content more. The occupation of a human being and the expose to harmful substances will decreases the level of sulphur by the diminution of sulphur proteins in the hairs. This is due to the damage of bonds by acids and alkaline groups leading to structural abnormalities in the hair. The N is a close range of 3.06 to 10.94 %. It is known that N is an important element in the fertilizing systems and plays vital role in irrigation and growth.

Degraded hair waste water as biofertilizer and dosage optimization

As per the patent KR101043568B1 submitted by South Korea in 2010, all kinds of human hair discarded in the beauty salon are used as raw materials and the waste material is recycled to completely form hydrophilic cuticle cell layers, cortical keratin proteins, melanin pigment molecules and medulla cytoplasm to be converted into liquid phase. The waste liquid after keratin extraction was applied on Okra (*Abelmoschus esculenthus*) seedlings for an interval of 90 days. The mode of application was fertigation and foliar spray on root, shoot and soil. Five treatments were designed as follows: Treatment 1:10 g of hair waste with 100 ml deionized water; Treatment 2: 20g hair waste with 100 ml deionized water; Treatment 3: 10 g of hair waste with 200 ml deionized water; Treatment 4: 20g hair waste with 200 ml deionized water; Treatment 5: Storage in shade (Control).

Physiochemical Composition of Human Hair Waste Water

The pH of the enriched waste water was near neutral to alkaline range of 8.0. The results revealed that the wastewater collected from the keratin extraction effluents contain pollution indicator parameters such as EC, pH, total solids, considerably higher than the tolerance limits recommended by the World Health Organization. The C:N ratio ranging from 20 % to 30 % is considered as optimum for plant growth. This suggests possible irrigation capacity of waste water along with 24.36 % of organic carbon.

Here in this study, from Table 2 it is evident that the macronutrient content of nitrogen is 0.12%, potassium is 1.763 %, calcium, magnesium and hydrogen are 4.158%, 2.749 % and 18.46% respectively. The micronutrient content is found to be reduced with Iron, Magnesuim and copper ranging from 3.14%, 0.085% and 0.090%. It relates to an organic fertilizer

composition which on adding to addition of healthy immobilized soil microorganisms inhabiting the humus of the plantation to convert the chemical structure of the hair hydrolyzable material into a biological useful material. This nutrient structure of hair waste water transforms the soil microbes to balance the optimum ratio of required fertilizer for plant.

Biometric Observations

Effect of different treatments on germination and vigour index of Okra

The data presented in Table 3 and Figure 6 indicated that germination percent in the range of 53.33 % to 86.67 % for *Abelmoschus esculenthus*. Effect of treatments on germination was found to be significant and the lesser the hair waste fertilizer, the more is the germination % compared to control plant with no fertilizers. This may be due to better mobilization of nutrients, hydrolyzation of reserved carbohydrates and better enzyme activity. This results in rapid degradation of proteins to aminoacids and finally initiation of embryo. The most important parameter in germination is the breaking of dormancy in recalcitrant seeds by the inhibition of water by the endosperm. Seedling vigour was also significantly influenced by the treatments. Since the vigour index is the product of seedling height and germination percent, the effect of treatments as depicted in Figure 7 were more pronounced in low concentration of hair waste sample. As the seedling height of T_2 is higher than that of T_4, the vigour index registered higher value for T_2 compared to T_4, highlighted as 968 and 845.33 respectively. This could be due to presence of beneficial microbial biomass and nutrient status in soil along with various growth promoting substances like hormones and enzymes.

Effect of treatments on seedling height at biweekly intervals of Okra

The effect of treatments on seedling height was evident as per the Figure 8 and Table 4. The highest seedling height (34.723 cm) at 90 days after planting was recorded in the treatment with lower hair content. The lowest seedling height in *Abelmoschus esculentus* was recorded for the treatment control.

Effect of treatments on seedling girth at biweekly intervals of Okra

Assessment of different treatments on seedling girth of Okra as in Figure 10 and Table 5 revealed that the treatments were significantly different during the initial two months but at 90 days after planting the highest

Sl.No	Parameter	Method
1.	pH	pH meter[38]
2.	Electrical Conductivity	EC meter[38]
3.	Total Nitrogen	Modified Kjeldhal digestion and distillation[39]
4.	Organic Carbon	Ashing method[38]
5.	Total Phosphorous	Diacid digestion and estimation by Vanodomolybdate yellow colour method[40]
6.	Total Potassium	Diacid digestion and Flame photometric determination.[39]
7.	Total Calcium and Magnesium	Diacid digestion and determination using inductively coupled plasma atomic emission spectroscopy (ICP-AES).
8.	Total Sulphur	Turbidimetric method[41]
9.	Total Iron, Manganeese, Zinc and Copper	Diacid digestion and determination using Inductively coupled plasma atomivc emission spectroscopy (ICP-AES)
10.	Heavy Metals	Diacid digestion and ICP-AES

Table 1: Methodology of residual hair water analysis, potting mixture and Okra seeds

Table 2: Physiochemical composition of human hair waste water.

Parameter	Human Hair Waste water values
Total solids (%)	1056
pH	8.0
Waste water EC(dS m^{-1})	1400
Organic carbon in waste water (%)	24.36
C:N ratio	20.85
N(%)	0.12
P(%)	1.7630
K(%)	BDL*
Ca(%)	4.158
Mg(%)	2.749
H(%)	18.46
Fe (%)	3.144
Mn(%)	0.085
Zn(%)	BDL*
Cu(%)	0.090

*BDL-Below Detectable Level

Table 3: Effect of different treatments on germination and vigour index of Okra.

Treatments	Germination (%)	Vigour index
T$_1$	66.67^{bc}	650.00^{bc}
T$_2$	86.67^a	968.00^a
T$_3$	60.00^c	578.00^c
T$_4$	80.00^{ab}	845.33^{ab}
T$_5$	53.33^c	480.00^c
CD(0.05)	16.271	249.03

T$_1$:10 g of hair waste with 100 ml deionized water; T$_2$: 20g hair waste with 100 ml deionized water; T$_3$: 10 g of hair waste with 200 ml deionized water;T$_4$: 20g hair waste with 200 ml deionized water; T$_5$: Storage in shade (Control)

DISCUSSION

While a significant amount of research has been carried out on several sources of keratin, there are scanty reports available on the extraction, characterization and utilization of keratin from bovine hooves, which is a significant source of keratin in comparison to other sources of keratin. We report the extraction and characterization of keratin from the much un-explored raw material-bovine hoof in the reduced form.[15] Keratin was obtained in the form of an aqueous solution from pulverized raw hooves using urea, Sodium dodecyl sulfate (SDS) and 2-mercaptoethanol at 60ºC. It is known that urea facilitates in breaking of non-covalent bonds,[8] SDS being a protein denaturant disrupts strong intermolecular interactions, while 2-mercaptoethanol cleaves the disulfide bonds in keratin, thereby increasing its solubility.[22] In keratin 2-mercaptoethanol cannot approach the site of the disulfide bonds, causing difficultly in reducing the disulfide bonds. Therefore, urea was used to increase the accessibility of the

growth was recorded in treatment 4 where foliar spray of 20 g hair waste with 200 ml distilled water is done.

Effect of treatments on number of leaves/seedling of Okra

The effect of treatments on number of leaves of seedling, were significant for 90 days after planting. As recorded in Figure 9 and Table 6 the highest number of leaves being recorded in T$_2$ at 90 days and the seedlings treated with dosage 20 g in 100 ml was found to be the best, recording the maximum number of leaves.

Table 4: Effect of treatments on seedling height at biweekly intervals of Okra.

Treatments	Seedling Height (cm)					
	15 DAP	30 DAP	45 DAP	60 DAP	75 DAP	90 DAP
T_1	15.173[b]	16.670[b]	18.397[c]	19.650[d]	26.620[b]	31.730[c]
T_2	16.477[a]	20.473[a]	22.407[a]	26.317[a]	26.577[b]	34.723[a]
T_3	12.677[c]	15.608[c]	17.767[d]	20.497[c]	23.620[c]	26.590[d]
T_4	15.847[ab]	20.743[a]	21.730[b]	21.720[b]	27.637[a]	32.813[b]
T_5	12.340[c]	14.307[d]	15.273[e]	16.397[e]	23.337[c]	27.430[d]
CD(0.05)	0.936	0.545	0.534	0.633	0.649	0.956

*DAP-Days after planting

T_1:10 g of hair waste with 100 ml deionized water; T_2: 20 g hair waste with 100 ml deionized water; T_3: 10 g of hair waste with 200 ml deionized water; T_4: 20 g hair waste with 200 ml deionized water; T_5: Storage in shade (Control)

Table 5: Effect of treatments on seedling girth at biweekly intervals of Okra.

Treatments	Girth (cm)					
	15 DAP	30 DAP	45 DAP	60 DAP	75 DAP	90 DAP
T_1	0.107[bc]	0.137[ab]	0.153[c]	0.193[b]	0.270[ab]	0.453[c]
T_2	0.140[a]	0.163[a]	0.200[a]	0.230[a]	0.287[a]	0.553[a]
T_3	0.127[ab]	0.157a	0.173[b]	0.193[b]	0.260[bc]	0.437[c]
T_4	0.143[a]	0.160[a]	0.183[ab]	0.230[a]	0.287[a]	0.507[b]
T_5	0.100[c]	0.120[b]	0.137[c]	0.163[c]	0.243[c]	0.373[d]
CD(0.05)	0.022	0.027	0.019	0.018	0.024	0.033

*DAP-Days after planting

T_1:10 g of hair waste with 100 ml deionized water; T_2: 20g hair waste with 100 ml deionized water; T_3: 10 g of hair waste with 200 ml deionized water; T_4: 20g hair waste with 200 ml deionized water; T_5: Storage in shade (Control).

Table 6: Effect of treatments on number of leaves/seedling of Okra.

Treatments	Number of leaves					
	15 DAP	30 DAP	45 DAP	60 DAP	75 DAP	90 DAP
T_1	4.497[c]	4.527[d]	4.947[e]	5.553[e]	8.223[e]	1.950[e]
T_2	6.273[a]	7.060[a]	7.543[b]	9.830[a]	13.433[b]	16.680[a]
T_3	5.093[b]	5.100[c]	6.800[c]	8.827[c]	12.760[c]	14.330[c]
T_4	6.287[a]	6.793[b]	8.070[a]	9.417[b]	13.910[a]	16.347[b]
T_5	4.060[d]	4.497[d]	5.260[d]	6.120[d]	9.337[d]	12.697[d]
CD(0.05)	0.057	0.085	0.074	0.187	0.385	0.042

*DAP-Days after planting

T_1:10 g of hair waste with 100 ml deionized water; T_2: 20g hair waste with 100 ml deionized water; T_3: 10 g of hair waste with 200 ml deionized water; T_4: 20g hair waste with 200 ml deionized water; T_5: Storage in shade (Control)

disulfide bonds to thiol by breaking the hydrogen bonds and thus swelling the keratin filaments.[23] This solution is expected to be stable for 1year when stored at 20°C-24°C.[24] The quantitative estimation of keratin was initially determined by Lowry and Bradford's method of protein estimation. But, the results shown by both these analyses were not accurate and constant. This might be due to the presence of some traces of β-mercaptoethanol in the final dialysate. As per a study conducted, neither Bradford nor Lowry's method is an accurate estimation technique.[25] Hence, protein content was determined using CHNS analyser.[26] The bands obtained are related to the low-sulfur keratin and were in line with the previous reports.[27] The buckling to the surface of the fibres after extraction as seen in the FE-SEM images, are consistent with the removal of cortical material from the hair fibres. Nevertheless, SEM provides high-resolution images with detailed
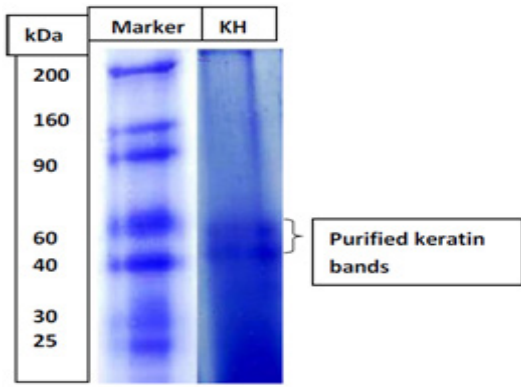
Figure 1: SDS-PAGE of standard protein molecular weight markers (left lane) and hair keratin (right lane) showing two bands around 45-50 and 55-60 kDa.
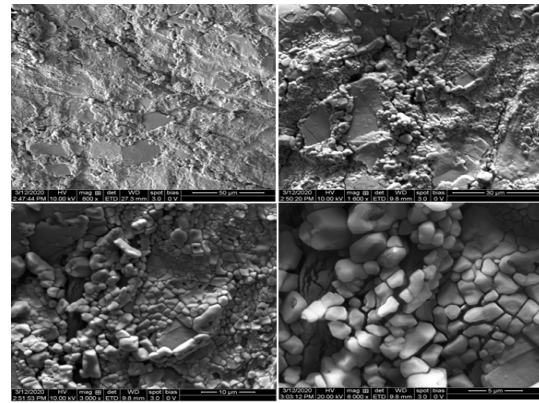

Figure 2: FESEM images of raw hair (from top left): Surface morphology of non-extracted hair shaft at (a) 400 μm (b) 100 μm (c) 50 μm (d) 20 μm.

Acquisition   Date:3/12/2020 3:32:09 PM HV:20.0kV   Puls th.:1.30kcps

El AN Series unn. C norm. C Atom. C Error (1 Sigma)
      [wt.%] [wt.%] [at.%]   [wt.%]

C 6 K-series 56.40 56.40 63.28   8.91
O 8 K-series 43.60 43.60 36.72   8.22

      Total: 100.00 100.00 100.00

Figure 3: EDAX Spectrum of hair before keratin extraction.

Figure 4: FESEM Images of hair after keratin extraction(from top left): Surface morphology of extracted hair shaft at (a) 50 μm (b) 30 μm (c) 10 μm (d) 5 μm.
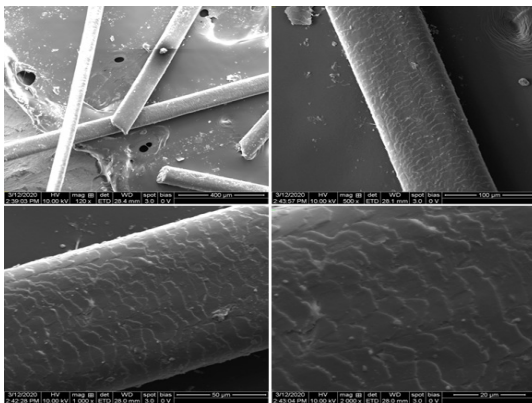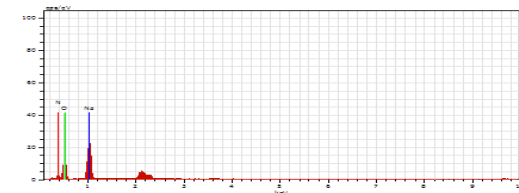

Acquisition   Date:3/12/2020 3:51:56 PM HV:20.0kV   Puls th.:8.38kcps

El AN Series unn. C norm. C Atom. C Error (1 Sigma)
      [wt.%] [wt.%] [at.%]   [wt.%]

O   8 K-series 13.44 48.09 53.97   2.06
Na 11 K-series 11.45 40.97 32.00   0.78
N   7 K-series   3.06 10.94 14.03   0.74

      Total: 27.94 100.00 100.00

Figure 5: EDAX Spectrum of hair after keratin extraction.
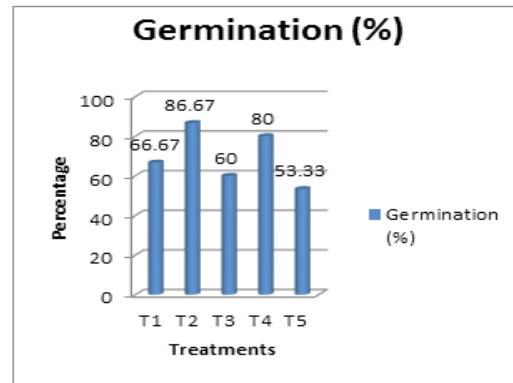

Figure 6: Effect of different treatments on germination percentof Okra.

surface information, as previously demonstrated in several studies on human hair.[28-31] In summary, SEM is a valuable tool to study overall human hair ultra structural properties. However, the present study aimed at protein identification and optimized extraction

methods of hair waste water after keratin extraction. It was found that from Table 2 during keratin extraction there was a reduction in total solids compared to the substrate hair. It may be due to the loss of nitrogen as amino acids on keratin extraction. The values 1056 are in
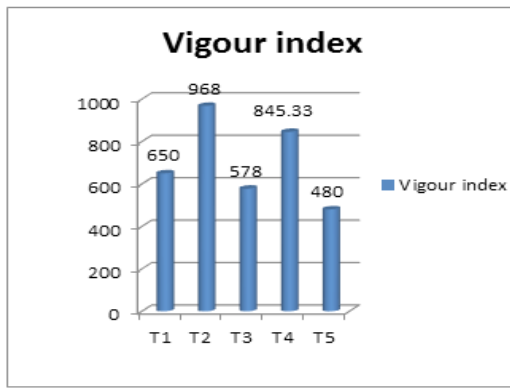
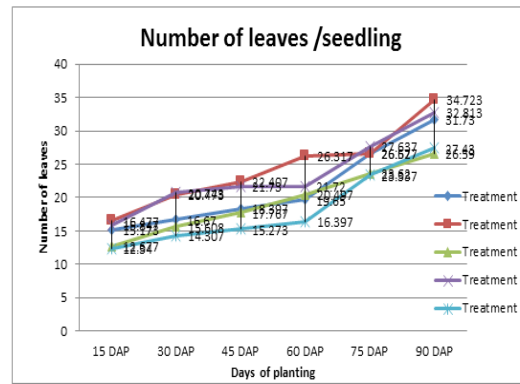**Figure 7: Effect of different treatments on vigour index of Okra.**

Figure 9: Effect of treatments on number of leaves/seedling of Okra.
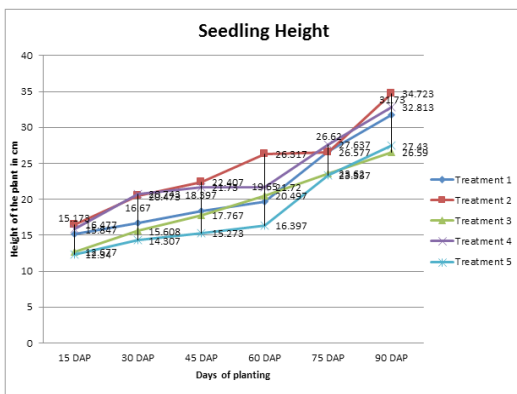

Figure 8: Effect of treatments on seedling height at biweekly intervals of Okra.
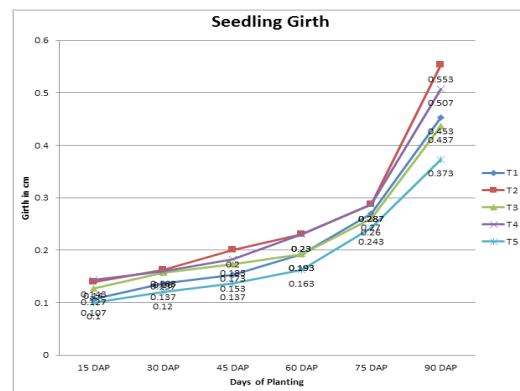

Figure 10: Effect of treatments on seedling girth at biweekly intervals of Okra.

accordance with that of a previous report.[32] Significant enhancement of germination was noticed for different waste water treatments with organics.[33] The application of spentwash diluted at higher levels (50 times) has increased germination percentage, growth fruit yield and fruit quality of Bhendi (*Abelmoschus esculanthus*) in a pot culture experiment.[34] Increased plant height in seedlings with foliar application of low hair waste fertilizer may be due to presence of ammonical nitrogen and other nutrients.[35] Seedlings of Okra are mainly used for grafting purpose as root stock for management and control of *Meliodogyne incognita* for which sufficient girth is an important prerequisite.[36] The supremacy of dosage concentration might be due to better root proliferation which is evident from the root weight of corresponding treatments.[37]

CONCLUSION

In summary, the solubility of human hair and the yield of keratin are dependent on the destruction of the cuticle layer and the fracture of disulfide bond. The extraction rate of keratin was 75.3% is reported. Out of 10 g of hair subjected to extraction, 6.8 g of keratin was extracted. The waste water collected was around 5.35 ml. Co-digestion of hair waste water after keratin extraction with seedlings of Okra is practically feasible for the generation of organic fertilizers with necessary nutrient content of fertilizing nature. By any chance the ratio of human hair waste water with neutral pH water application should not be lower than 2:1. If we use hair waste water alone for fertilizing plants, the soil bacteria will become inactive due to high alkalinity. Since the hair waste water treatment are found to be effective than control plants, any of the combination would be resorted for better root stock production in Okra. We can link all the findings for the better economic gains by the agro entrepreneurs.

ACKNOWLEDGEMENT

and technology, Bharathiar University, Coimbatore for helping with analytical works and experiments. We also thank Department of Agricultural Microbiology, Kerala Agricultural University, Thrissur for their inspiration and good wishes to carry out this study.

Funding

CONFLICT OF INTEREST

There is no conflict of interest.

ABBREVIATIONS

DMEM: Dulbecco's Modified Eagle's medium; **DCM:** Di cholro methane; **SDS:** Sodium Dodecyl Sulphate; **KBr:** Potassium bromide; **CHNS:** Carbon Hydrogen Nitrogen Sulphur Analyzer; **KH:** Hair Keratin; **EDAX:** Energy Dispersive X-Ray Analysis; **EC:** Electrical Conductivity.

REFERENCES

1. Kornillowicz K, Bohacz J. Biodegradation of keratin waste: Theory and practical aspects. Wate Manag. 2011;31(8):1689-701.

2. Debicki R, Rejiman J, Wontroba J. Effect of keratin bark urea granulates on the physiochemical properties of soil. Prob Notes Adv Agri Sci. 1989;370:39-48.

3. Dechnik I, Wiater J. Effect of fertilization with various organic materials on the chemical composition of rye during the tilling and flowering phases. Prob Notes, Adv Agri Sci. 1989;70:119-28. (in Polish).

4. Nurynski J. The usability of keratin bark urea granulates in fertilizing greenhouse tomatoes. Prob notes Adv Agric Sci. 1989;370:237-43. (in Polish).

5. Wolski T, Orlikowski LB, Glinski J. The use of processed lignin-cellulose and kertin waste in plant protection. Prob Notes Adv Agric Sci. 1996;437:359-64.

6. Powlowski F, Wesolowski M, Styk B. The usability of keratin bark urea granulates in weeding grains and *Brassica napus* var, *Arvensis* f. *Annua*. Prob Notes Adv, Agri Sci. 1989;370:217-28. (in Polish).

7. Gostkowask K, Woytoxic ZB, Szember A, Furczak J, Jezieska-Tys S, Jaskiewicz WM. Effect of various fertilizers on the microbiological activity of sandy soil. Prob Notes Adv Agri Sci. 1989;370:75-84. (in Polish).

8. Li M, Zhang H, Wang JX, Pan Y. A new essential protein discovery method based on the integration of protein-protein interaction and gene expression data. BMC Systems Biology. 2012;6(15):2-9.

9. Tranv CD, Mututuvari TM. Cellulose, chitosan and keratin composite materials; facile and recyclable synthesis, conformation and properties. ACS Sustainable Chemistry and Engineering. 2016;4(3):1850-61.

10. Idris A, Vijayaraghavan R, Patti AF, MavFarlose DR. Distillable Protic Ionic Liquids for keratin dissoloution and recovery. ACS Sustainable Chemistry and Eng. 2014;2(7):1888-94.

11. Akhtar W, Edwards M, Farwell W, Nuthrown M. Fourier transforms Raman spectroscopic study of human hair Acta Part A. J Spectrochem. 1997;53(7):1021-31.

12. Stephan R. Films based on human hair keratin as substrates for cell culture a tissue engineering. J Biomater. 2009;30(36):6854-66.

13. Marian AN, Francis O, James HE, David DW, Luke PMT. Characterization of beauty sloon waste water from Kwame Nkrumah University of Science and Technology, Kumasi, Ghana and its surrounding communities. Environ Health Insight. 2006;10(1):147-54.

14. Sidra Y, Mujeeb UR, Ihsan A, Rehmat I, Fazal MK, Amir U, *et al*. Bio mineralization of iron ore tailing soil with iron oxide bacteria and *Sesbania sesban* plant. Bulletin of Environ: Pharma and Life Sci. 2019;9(1):33-42.

15. Yamauchi K, Yamuchi A, Kusanoki T, Kohda A, Konish Y. Preparation of stable aqueous solution of keratins and physiochemical and biodegradation properties of films. J of Biomed Res. 1996;31(4):439-44.

16. Neuhoff V, Reinhard S, Hansjorg E. Clear background and highly sensiyive protein staining with Coomassie Blues dyes in polyacrylamide gels, a systematic analysis. Electrophoresis. 1985;6(9):427-48.

17. Jiang SQ, Zhang L, Haixia Wu, Gang C. Study on effective extraction of keratin from human hair wastes. Integrated Ferroelectrics. 2017;180(1):102-7.

18. Helge RA, Hao L, Hubert L, Marcus M, Kristian P, Annelies SF, *et al*. Supernumerary human hair cells-signs of regeneration or impaired development: A field emission scanning electron microscopy study. Ups CJ Med Sci. 2017;122(1):11-9.

19. Gurav RG, Jadhav JP. A novel source of biofertilizer from feather biomass for banana cultivation. Environ Sci and Poll Resear. 2012;20(7):4532-9.

20. Sadasivom S, Manikan A. Biochemical methods for agricultural sciences (8th Ed.) Wiley Eastern Ltd. New Delhi and Tamil Nadu Agricultural University, Coimbatore. ;422.

21. Panse VG, Sukhatme PV. Statistical, methods for agricultural workers (4th Ed), Indian Council of Agricultural Research, New Delhi, India. 1985;347.

22. Bragulla HH, Homberger DG. Structure and function of keratin protein in simple, stratified, keratinized and cornified epithelia. J Anat. 2009;214(4):516-59.

23. Strnad D, Uschov V, Deber C, Grater F, Parry DAD, Omary MB. Unique aminoacid signatures that are evolutionary conserved distinguish simple-type, epidermal and hair keratins. J Cell Sci. 2011;124(24):4221-37.

24. Srinivasan B, Kumar K, Shanmugan K, Sivagnam UT, Reddy NP, Senghal PK, *et al*. Porous keratin scaffold-promising biomaterial for tissue engineering and drug delivery. J Biomed Mater Res-Part B. 2010;92B(1):5-12.

25. Okutucu B, Dincer A, Habib O, Zihnioglu F. Comparison of five methods for determination of total plasma protein concentration. J of Biochem and Biophysicl Meth. 2007;70(5):709-11.

26. Gonzalez L, Garcia CV, Fernadez FGA, Bustos CG, Chisiti Y, Sevilla JMF. Protein measurement of microalgal and Cyanobacterial biomass. Bioresource Technol. 2010;101(19):7587-91.

27. Fraser RD, Parry DA. The molecular structure of reptilian keratin. Int J Biol Mcromol. ;19(3):207-11.

28. Kaliyadan F, *et al*. Scanning electron microscopy study of hair shaft damage secondary to cosmetic treatments of the hair. Internat J of Tri. 2016;8(2):94-8.

29. Echeveria XP, Romero WA, Carreno NR, Zegpi MS, Gonzalez SJ. Hair shaft abnormalities, Pilli bifurcate, a scanning electron microscopy analysis. Paediatric Dermatology. 2009;26:169-70.

30. Akhtar W, Edwards M, Farwell W, Nuthrown M. Fourier transforms Raman spectroscopic study of human hair. J Spectrochem Acta Part A. 1997;53(7):1021-31.

31. Mazotto AM, *et al*. Keratinolytic activity of *Bacillus subtilis* AMK using human hair. Letters in Applied Microbiology. 2010;50(1):89-96.

32. Marian AN, Francis O, James HE, David DW, Luke PMT. Characterization of beauty sloon waste water from Kwame Nkrumah University of Science and Technology, Kumasi, Ghana and its surrounding communities. Environ Health Insight. 2006;10:147-54.

33. Rasmala N, Ynoussa M, Razacswende FQ, Wendpoure GBK, Badova B, *et al*. Effect of watter quality on the germination of Okra (*Abelmoshus esculenthus L)* seeds. Int J of Agro. 2009;1-7.

34. Soundarrajan M, Pitchai GJ. Impact of distillery spent wash on seed germination, seedling growth and yield of Bhendi (*Abelmoschus esculenthus* L). J Ecobiol. 2007;385-90.

35. Rahman IU, Shamsher A, Inayat R, Muhammad A, Muhmmad I, Nauman S, *et al*. Effect of seed priming on growth parameters of Okra (*Abelmoschus esculenthus* L). Pure App Biol. 2016;5(1):165-71.

36. Edgard HCS, Rento SS, Guilherme MD, Carolin AF, Marcus VM, William SC, *et al*. Grafting as a management tool to control *Meloidogyne incognita* in Okra: Identifying root stocks candidates. Sci Horti. 2019;246:354-9.

37. Egbenni UPO, Okolie PN, Adejuyitan OE, Sobande AO, Akinyemi O. Effect of industrial effluents on the growth of anatomical structures of *Abelmoschus esculentus* (Okra). Afri J of Biotech. 2009;8(14):3251-60.

38. The Fertilizer Control Order. Ministry of Agriculture and rural development (Department of Agriculture and Cooperation), New Delhi. 1985.

39. Jackson ML. Soil Chemical Analysis. Soil Science, University of Wisconsin, USA, Madison. ;89-102.

40. Piper CS. Soil and plant analysis. Ham Publication, Bombay. 1966;236.

41. Bhargava BS, Raghupathi HB. Analysis of plant material for macro and micronutrients, Methods of analysis of soils, plants, water and fertilizers, Mlhotra Publishing house, New Delhi. 1995;61-2.

Cite this article: Unnikrishnan G, Ramasamy V. Extraction of Keratin from Human hair with Production of Biofertilizer from Waste Liquid of Hair Extraction and its Efficient Application on Growth Yield of *Abelmoschus esculentus* L. Asian J Biol Life Sci. 2020;9(2):119-28.