


Research

Evaluation and formulation of *Spirulina platensis* proteins for potential applications in hair care products

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Abstract

Spirulina platensis, a blue-green algae belonging to family Phormidiaceae, is well-known for its unique nutritional profile and high protein content. This study aims to isolate its proteins, formulate them into a cream and evaluate its potential as a hair treatment. *S. platensis* were collected from Nuweiba, Egypt and its protein was extracted, analyzed and used to prepare a cream. The cream was applied on natural hair strands compared to a control cream containing no protein. Both hair strands groups were observed via Scanning Electron Microscopy (SEM), Transmission Electron Microscopy (TEM), Atomic Force Microscopy (AFM) and evaluated by Fourier-transform infrared spectroscopy (FTIR), Raman spectroscopy, as well as, thermal analysis tests including thermogravimetric analysis (TGA) and Differential Scanning Calorimetry (DSC). The SEM images of the test group showed more intact cuticle with the absence of cracks and holes, also, the TEM images showed stronger non-porous cuticle compared to the control group. The AFM images of the test group was smoother with lower roughness average and step height compared to the control group. On the other hand, the raman spectra showed different band intensities for disulfide bonds and C-S bonds as well as increased number of β sheet conformations in the test group which is responsible for the smoothing effect. The thermal analysis tests revealed a clear difference between both groups as an indication of improved keratin structure on the test group. *S. platensis* proteins could be introduced as a potential candidate for hair care products.

Article Highlights

- The study evaluated *Spirulina platensis* protein as a potential candidate for hair treatment.
- The protein was extracted and formulated into a cream for testing on natural hair strands.
- The results showed that the cream improved the condition of hair strands, indicating that *S. platensis* protein could be adopted as a promising ingredient in hair care products.

Keywords Atomic force microscopy · Differential scanning calorimetry · Fourier-transform infrared spectroscopy · Raman spectroscopy · Scanning electron microscopy · Thermogravimetric analysis · Transmission electron microscopy

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1 Introduction

Spirulina platensis is the common name of the biomass of *Arthrospira platensis*. It belongs to the photosynthetic bacteria cyanobacteria (blue-green algae), family Phormidiaceae [1]. It is considered one of the richest food sources as it contains over 100 nutrients that are rarely found in any other grain, plant or herb. *S. platensis* contains a variety of fatty acids such as palmitic acid and vitamins such as vitamin E, vitamin B, biotin and folic acid. In addition to, a fairly high amount of minerals such as calcium. Besides, the reported HPLC screening revealed that it contains polyphenols and flavonoids. It also contains phytopigments such as xanthophyll, beta-carotene, phycocyanin and chlorophyll [2]. These interesting phytochemical constituents accounts for its employment as antioxidant, anti-inflammatory, immunomodulatory [3]. However, the main *S. platensis* nutrient is protein which represents approximately 61.57 g/100 g dry weight and makes it one of the richest protein sources. Owing to this, spirulina is known as “superfood” and the US Food and Drug Administration (FDA) allows its use as a food additive [4]. Its proteins are formed of a variety of essential and non-essential amino acids, especially; alanine, histidine, phenylalanine, cysteine, proline and l-glycine in high percentages which are proven to be beneficial for hair health by enhancing the moisture content and strength of hair, being the building blocks of naturally occurring protein in the hair particle. Specifically, alanine increases hair hydrophobicity while phenylalanine and histidine increase hair tensile strength. Likewise, cysteine increases the formation of disulfide bonds improving the mechanical, thermal, and chemical strength of hair. Moreover, proline aids in the formation of collagen, glycine is a hydrating amino acid, and aspartic acid is the building block of elastin and collagen [5].

Most of the protein-based commercial hair products contain proteins extracted from animal sources such as; keratin, collagen, and silk proteins that are mostly prepared by unethical procedures, non-ecofriendly and might transfer zoonotic diseases [6]. Thus, researchers have been interested in using plant-based nano-medicines for promoting hair health [7]. However, plant sources like wheat and vegetable proteins contain insufficient amounts of amino acids and have high incidence of causing several allergic reactions [8].

Based on the aforementioned facts, this study aims to formulate *S. platensis* proteins in a hair cream and evaluate the potential of this formulation to be used in hair care products.

2 Material and methods

2.1 Material

2.1.1 *S. platensis* algae

S. platensis algae was collected from Nuweiba, Egypt. It was kept in water tanks where water was pumped from one tank into another with mesh sheets attached to the top of the second tank. The water was separated from the biomass in the second tank and then extracted on two stages, the first stage using the 45-micron mesh filter and then the obtained filtrate passed through the 25-micron mesh filter. The algae were then transported in opaque, airtight containers and kept in a cool dry place prior to use.

2.1.2 Experimental hair samples

20 gm of donated brown hair of type 2C, which is wavy with well-defined S-shaped waves was obtained, thoroughly washed using an adequate amount of shampoo, massaged for 30 min to get rid of any remains, then rinsed under water for 1 min then they were left to dry at room temperature until damp. The dried hair was then divided equally into two groups (test and control).

2.1.3 Chemicals and reagents

Sodium hydroxide, hydrochloric acid, hexane, potassium hydroxide, glycerine, stearic acid, glutaraldehyde, osmium tetroxide, alcohol, epoxy resin, uranyl acetate, lead acetate and potassium bromide were purchased from Sigma-Aldrich (St Louis, MO).

2.1.4 Instrumentation

Centrifuge, autoclave, probe sonicator (Biologics, Inc., VA, USA) and high-pressure homogenizer (Constant systems LTD., UK) were used for protein extraction. For Amino acids determination Sykam Amino Acid Analyzer (Sykam GmbH, Germany) equipped with Solvent Delivery System S 2100 (Quaternary pump with flow range 0.01 to 10.00 ml/min and maximum pressure up to 400 bar). Autosampler S 5200, Refrigerated Reagent Organizer S 4130 (GRAM, Denmark), Oven and rotary evaporator. Scanning Electron Microscope (Model: Jeol JSM-5200), SPI-MODULE sputter coater, Transmission Electron Microscope (JEM-1400 TEM), Tolodin blue 1 X, Camera Lica ICC50 HD, CCD camera model AMT, Atomic Force Microscopy (Agilent 5600LS AFM, USA), IRAffinity-1 Fourier transform infrared spectrometer (Shimadzu, Kyoto, Japan), DTGS KBr Detector, NICOLET 380 FT, LabRAM HR Evolution (Confocal Raman Microscope), DTG-60H and DSC-60 were used for hair samples analyses.

2.2 Methods

2.2.1 *S. platensis* protein extraction

S. platensis biomass was solubilized at alkaline pH using 1 M NaOH followed by precipitation from the supernatant (obtained from the previous step) at acidic pH using 1 M HCl. The solid–liquid separation after the solubilization and the precipitation steps was achieved by centrifugation at 8670 for 35 min. The pellet and the supernatant from the solubilization step are henceforth referred to as alkali pellet and alkali supernatant, respectively, and those from the precipitation step are referred to as acid pellet and acid supernatant, respectively. The acid pellet was the protein isolate. The combined fraction of the alkali pellet and acid supernatant was the residual biomass [9]. A 6% slurry of *S. platensis* biomass was subjected to autoclaving, ultrasonication, and high-pressure homogenization. Autoclaving was carried out at 121 °C with 103.4 kPa (15 psi) for 30 min. Ultrasonication was carried out using a probe sonicator at 20% maximum power for 60 min. High-pressure homogenization involved two passes through a high-pressure homogenizer at 103.4 MPa (15 kpsi). The samples were placed on ice bath during ultrasonication and high-pressure homogenization, and a chiller was attached to the latter unit to minimize sample heating. The sample was subjected to protein solubilization at pH 11 for 60 min followed by solid–liquid separation. The total protein content of the samples was determined by kjeldahl method [10].

2.2.2 *S. platensis* protein analysis

The amino acids of *S. platensis* protein were estimated by adopting the method of Maehre et al. by the following procedure [11]:

2.2.2.1 Standard preparation A standard stock solution containing 15 amino acids (aspartic acid, threonine, serine, glutamic acid, glycine, alanine, cystine, valine, methionine, isoleucine, leucine, phenylalanine, histidine, lysine, arginine) was prepared. All amino acids concentrations were of 2.5 μMol/ml concentration, except cystine which was 1.25 μMol/ml. 60 μl were diluted in 1.5 ml vial with sample dilution buffer then filtered using 0.22 μm syringe filter then 100 μl was injected.

2.2.2.2 Sample preparation 100 mg of the protein sample was mixed with 5 mL hexane. The mixture was allowed to macerate for 24 h. Then, the mixture was filtered on whatman no. 1 filter paper and the residue was transferred into a test tube where it was incubated in an oven with 10 mL 6N HCl for 24 h at 110 °C. After the incubation, the sample was

filtered, evaporated on rotary evaporator and dissolved completely in 50 ml dilution buffer then diluted in a dilution buffer with ratio 1:20, then filtered using 0.22 µm syringe filter and 100 µl was injected.

2.2.3 Formulation of the hair cream

The aqueous phase formed of potassium hydroxide, glycerine and deionized water was dissolved in a water bath at a temperature of 80 °C. Stearic acid was dissolved in the water bath to form the oily phase. The temperature was maintained at 80 °C during adding the aqueous phase to the oily phase portion-wise with levigation after each portion. After being emulsified for 30 min, the cream was equally divided into 2 groups, test and control. Solely to the test group, the *S. platensis* protein extract was added. Both creams were allowed to cool naturally to room temperature.

2.2.4 Application of the cream on the hair

Test cream was applied on the test hair strands by using a brush from the top to the bottom in a homogenous way and was massaged for 30 s. The same procedure was applied on the control hair strands using the control cream. Both hair groups were left for 5 min and then rinsed for 1 min very well. This method was done every 3 consecutive days for 9 days, and then the hair strands were subjected to several analyses [12].

2.2.5 Transmission electron microscopy (TEM) and atomic force microscopy (afm)

Hair samples of both groups were separately sliced into approximately 1 mm slices and processed for TEM by fixation in glutaraldehyde and osmium tetroxide, then dehydrated in alcohol and embedded in an epoxy resin. Microtome sections was prepared at approximately 500–1000 micro meter thickness with a Leica Ultracut UCT ultramicrotome and the thin sections were stained with toluidin blue (1X) to be examined by camera Lica ICC50 HD. Additionally, Ultra-thin sections were prepared at approximately 500–1000 micro meter thickness and were stained with uranyl acetate and lead acetate, and then examined. Images were captured by CCD camera model AMT, optronics camera with 1632 × 1632 pixel format as side mount centrifugation, and this camera uses a 1394 fire wire board for acquisition [13].

2.2.6 Fourier transform infrared spectroscopy (FTIR)

The FTIR spectra of both hair samples were separately recorded over the range of 400–4000 cm^{-1} . Potassium bromide pellets containing the hair samples were prepared prior to FT-IR analysis (2 mg sample/300 mg KBr). First the accessory for analyzing the sample was prepared with a smart performer accessory that has the proper ATR crystal and the sample plate in FTIR was added. Sample was placed in the center of crystal directly under the pressure point. Then the pressure control ring was rotated clockwise until the pressure point contacts the sample. Rotating is continued until the sample is pressed firmly against the crystal surface then scan to collect a sample spectrum [14].

2.2.7 Raman spectroscopy

Raman spectra were obtained for both hair samples using a wavelength of 785 nm at an operating laser power of 50 mW to result in an excitation. Test and control hair samples were separately fixed to glass microscope slides to ensure hair straightening without stretching [15].

2.2.8 Thermogravimetric Analysis (TGA)

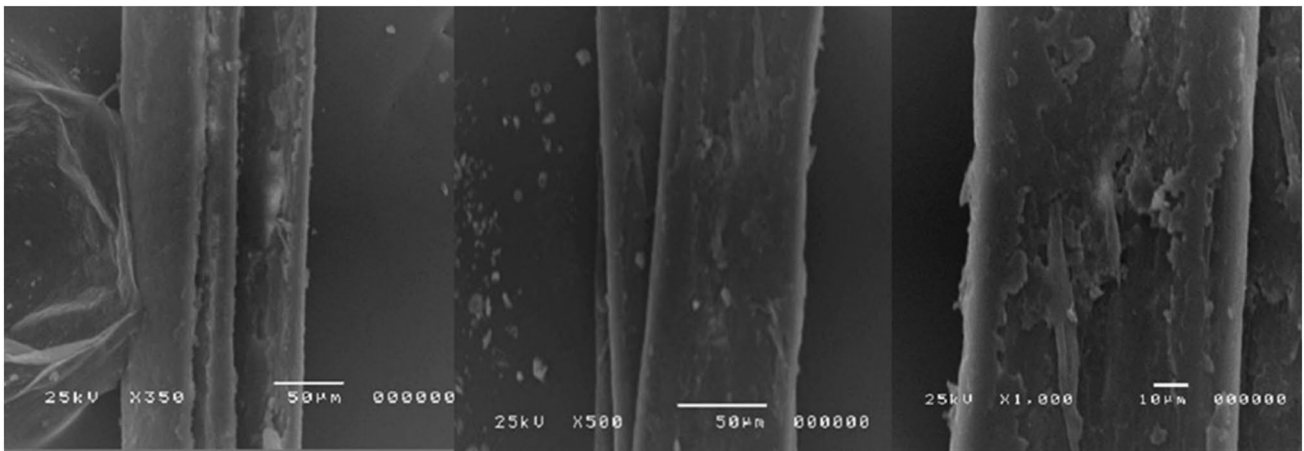
Each sample is added separately to the detector which is adjusted with rate 10 °C/min, and temperature is between 30 and 300 °C. The mass measurements were obtained in multiple times with the changes in temperature. A thermogram is produced which is a plot of mass against temperature [16].

2.2.9 Differential scanning calorimetry (DSC)

Each sample is added separately two the device which is adjusted at temperature between 30 and 300 °C. The heat flow from the furnace inside the DSC-60 to the sample is measured relative to the heat flow to reference material and

Table 1 The results of amino acids analysis of *S.platensis* protein

	Amino acid	Retention time [min]	Concentration [mg/100 mg]
1	Aspartic acid	8.923	0.024
2	Threonine	11.131	0.006
3	Serine	11.917	0.016
4	Glutamic acid	14.232	0.041
5	Glycine	20.363	0.023
6	Alanine	21.947	0.037
7	Cysteine	23.160	0.010
8	Methionine	24.765	0.020
9	Valine	27.531	0.006
10	Isoleucine	29.552	0.005
11	Leucine	31.269	0.018
12	Phenylalanine	33.411	0.039
13	Histidine	34.453	0.064
14	Lysine	39.307	0.009
15	Arginine	43.083	0.011

**Fig. 1** The SEM images of control hair group at resolutions 350 \times , 500 \times and 1000 \times

the amount of heat required to increase the temperature of the sample and the reference is measured as function of temperature. The DSC works by maintaining the same temperature in both the sample and the reference throughout the experiment [16].

3 Results

3.1 *S. platensis* protein analysis

Amino acids analysis was performed to qualitatively and quantitatively assess the amino acids content of *S. platensis* protein. The results shows that *S.platensis* protein is enriched with 8 essential amino acids such as leucine, isoleucine, lysine, phenylalanine, threonine, histidine, methionine and valine as well as 7 non-essential amino acids including glycine, glutamic acid, aspartic acid, alanine, serine, arginine and cysteine. Table 1 shows that the most abundant amino acid in *S.platensis* protein is histidine with concentration 0.064 mg/100 mg. It also shows relatively high concentrations of glutamic acid, phenyl alanine, alanine, aspartic acid, leucine, arginine and valine which reached (0.041, 0.039, 0.037, 0.024, 0.018, 0.011 and 0.006 mg/100 mg) respectively.

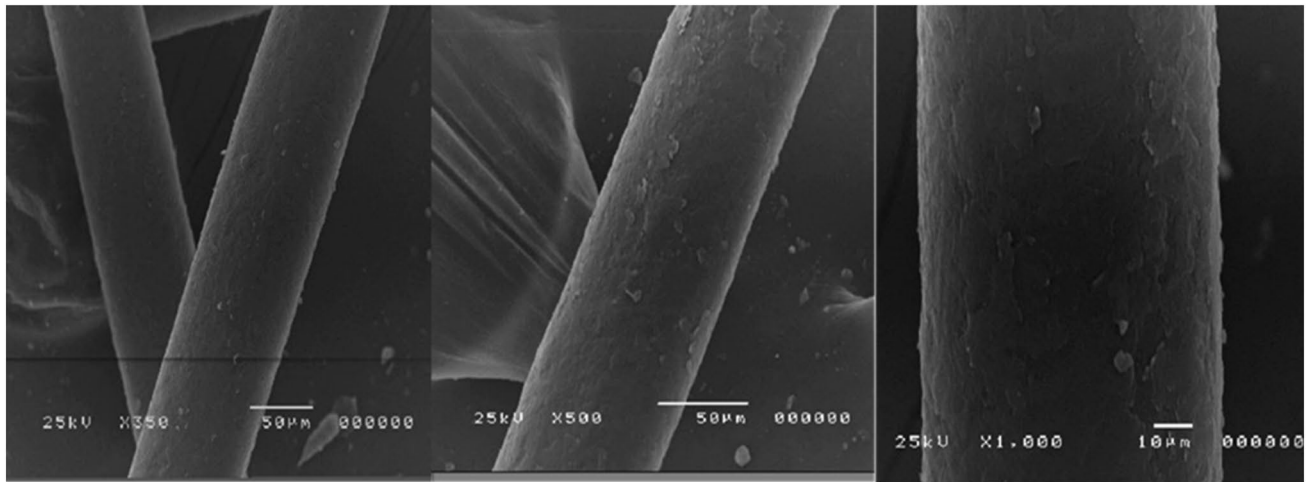


Fig. 2 The SEM images of test hair group at resolutions 350x, 500x and 1000x

3.2 Scanning electron microscopy

The hair samples images obtained at different resolutions showed a clear difference between test group and control group in the general appearance of the hair strands. The control group at resolution 350x showed multiple cracks along the hair shaft and at resolution 500x the cuticle was damaged that the regular and overlaying pattern were absent. Moreover, at resolution 1000x, the presence of holes inside the hair shaft can be seen clearly and the exposure of the cortex layer beneath the cuticle layer. On the other hand, the test group at resolution 350x and 500x the hair shaft was more intact than the control group with no visible damage or change to the hair cuticle. At resolution 1000x, minimal irregularity of the hair cuticle can be seen however there were no cracks or holes on the hair shaft (Figs. 1 and 2).

3.3 Transmission electron microscopy

Upon the evaluation of the changes of the ultra-structure, morphology, moisture content, and hair appearance by TEM, it was found that the test hair group is more moisturized, shinier, intact and healthy than the control group as shown in Figs. 3 And 4. The TEM examination of the hair strands of the test group shows the inner structure of the strand showing the cortex, endo-cuticle, exo-cuticle, and melanin which is carried by the cortex. The cuticle looks healthy, intact, sealed, and with low porosity, also, its lipid layer was balanced. In addition, the cortex, which is the main carrier of melanin, lipids, fibrous materials and keratin, was closed and well distributed with straightened ends. However, the control hair group

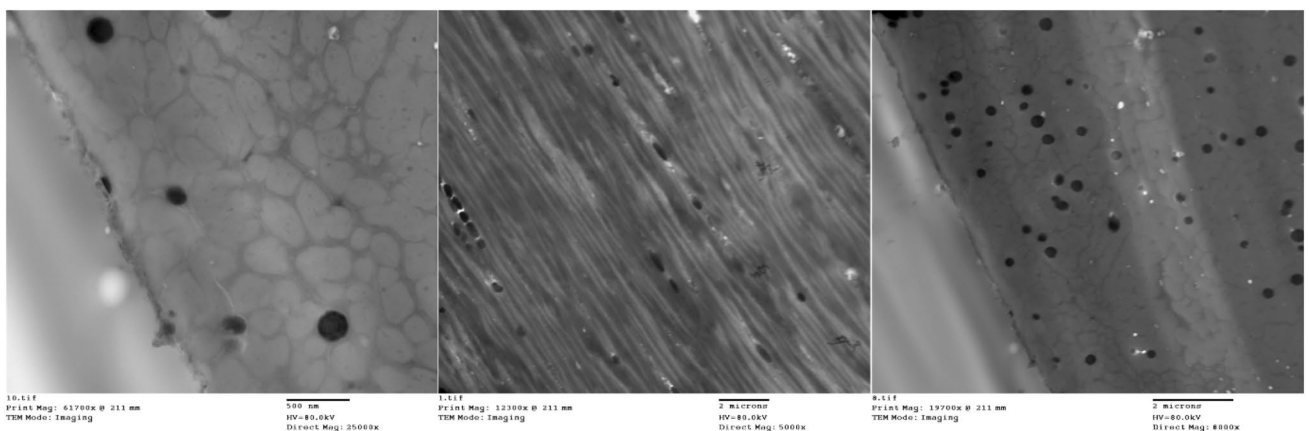


Fig. 3 Cross sectional and longitudinal images of control hair group by TEM

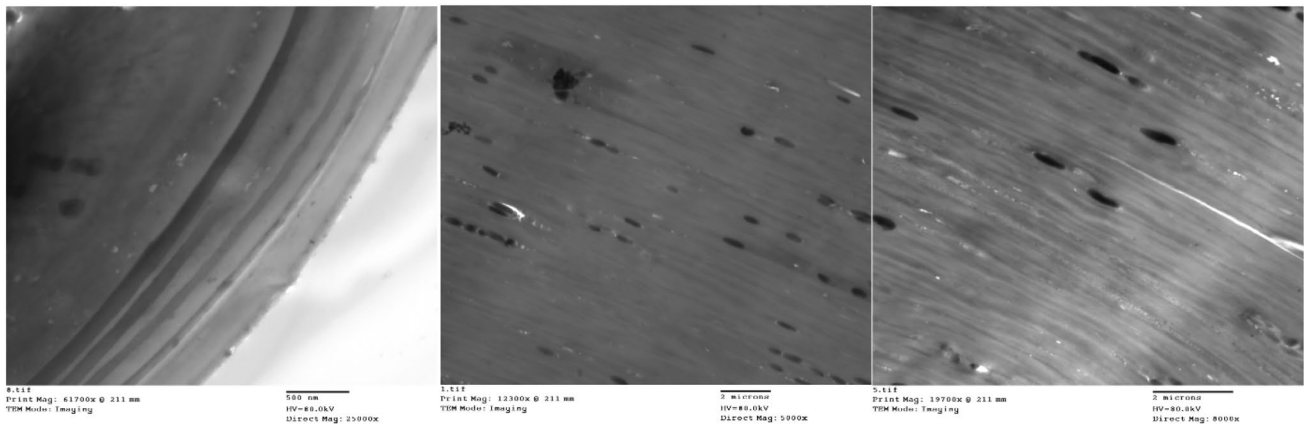


Fig. 4 Cross sectional and longitudinal images of test hair group by TEM

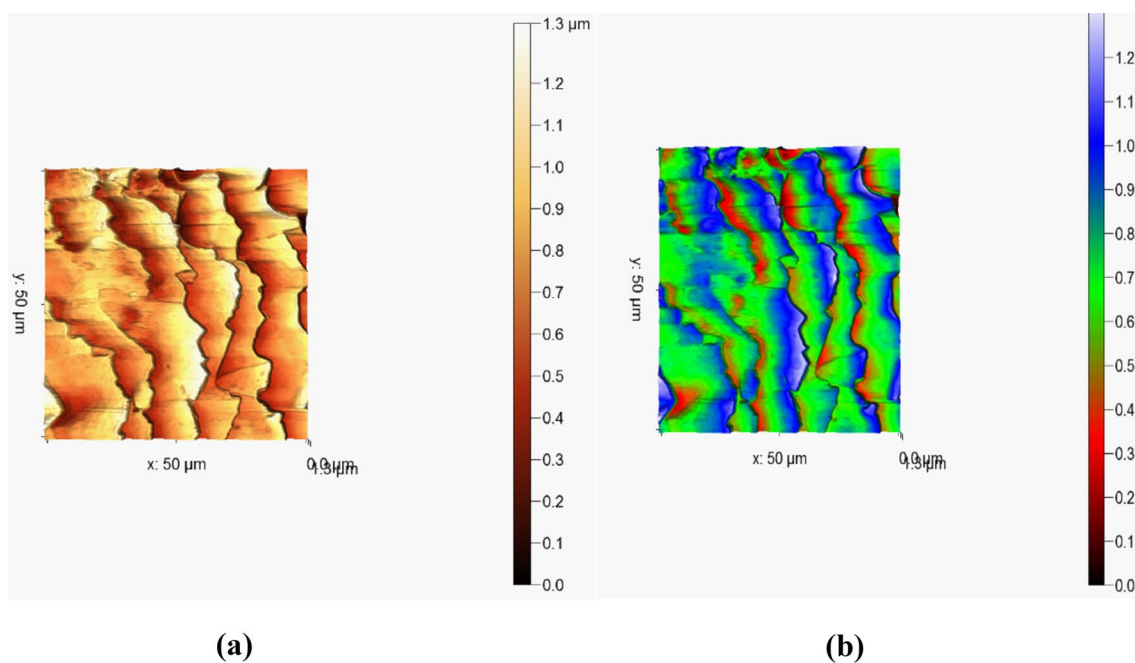


Fig. 5 AFM images of the control hair strands (image size $50\ \mu\text{m} \times 50\ \mu\text{m}$)

shows a clear damage in the outermost cuticle layer with multiple cracks seen on it, in addition to a rough, dull, extra porous and raised cuticle which leads to the destruction of lipid layer. Besides, the cortex is obviously damaged, tangled and contains many split ends with broken strands which will lead to keratin loss.

3.4 Atomic force microscopy

The AFM was used to investigate the details of the morphological changes of the hair after the application of the hair cream. As shown in Figs. 5, 6, 7, 8 that the outer layer of the hair is the cuticle that exhibits overlapping consecutive sheaths which are layers of imbricated and keratinized cells that form the cuticle. The images show a great difference between the control and test hair groups. Figures 5 and 7 of the control group shows cracking and miscellaneous damage with rougher surface than the test hair group in Figs. 6 and 8 in which the cuticle surface was smoother and intact. There are scale edges mainly of smooth contour, scale edge ghost and striated surfaces in Figs. 5 and 7, while there are

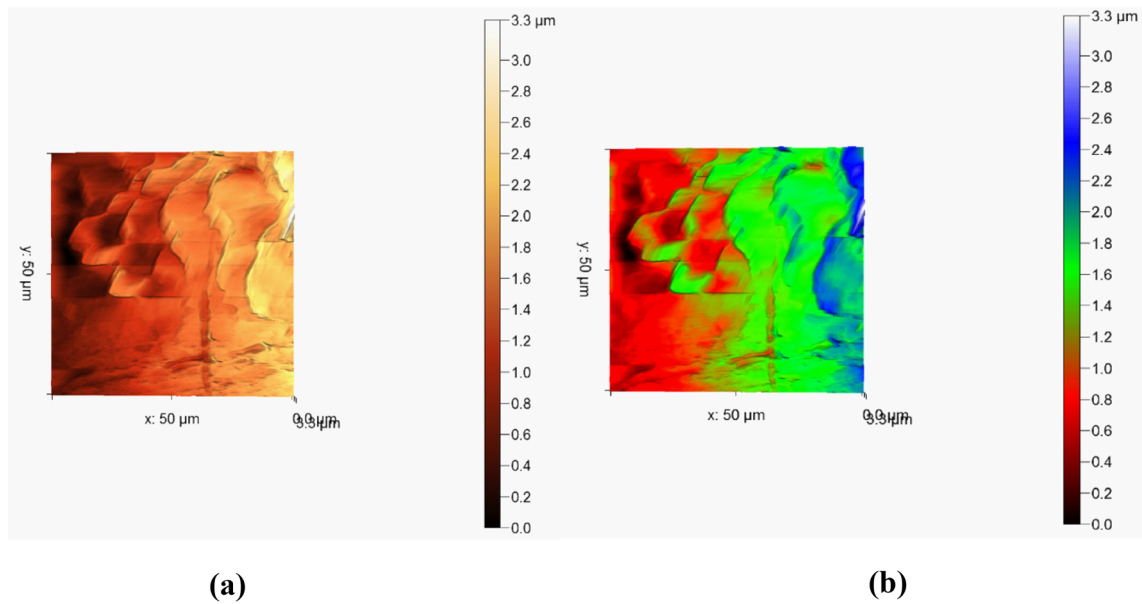


Fig. 6 AFM images of the test hair strands (image size 50 μm × 50 μm)

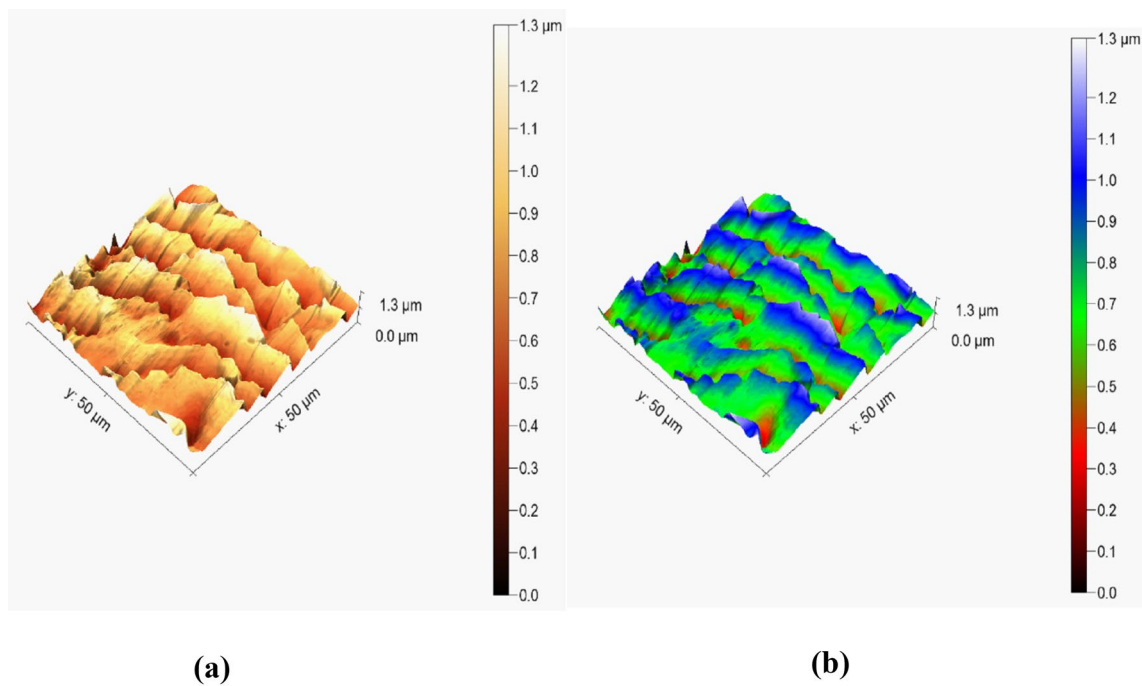


Fig. 7 3D-AFM images of the control hair strands (image size 50 μm × 50 μm)

few contoured scale edges remaining but ample evidence of scale edge ghosts and extent of surface striations in the test group (Figs. 6 and 8).

Moreover, as evident from Fig. 9 of the control hair group the roughness average is 43.5 nm which is much higher than the test group that is 16 nm as shown in Fig. 10. The edge of the steps was more uneven and rough in the control group (Fig. 9) while it was more smooth and regular in the test group (Fig. 10). Concerning the sheath heights, it was found that the height of one sheath relative to the next was higher in the control than the test hair as the maximum height in the control group was 336.5 nm while that of the test was 259.6 nm.

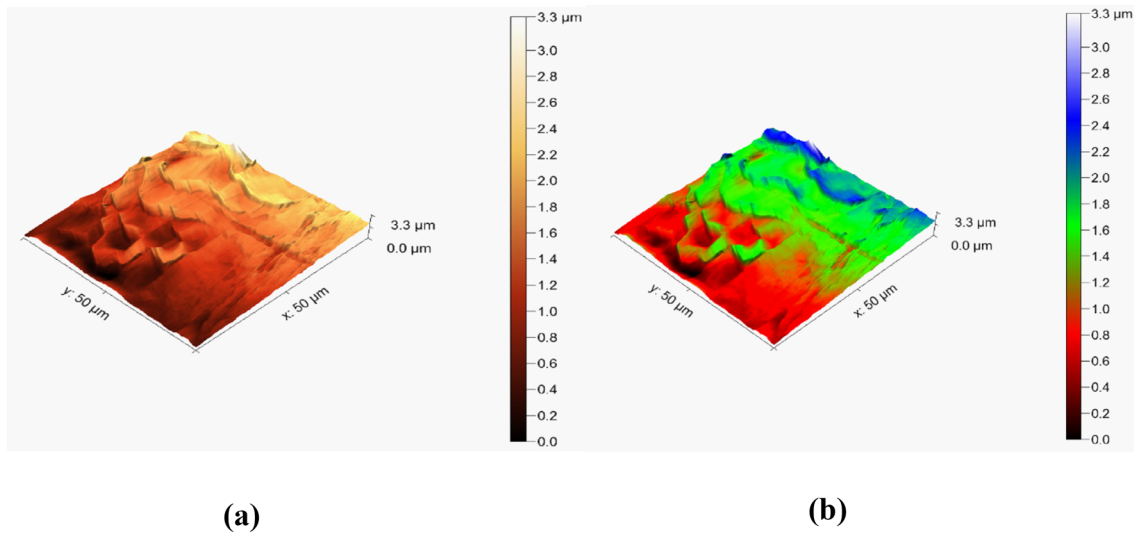


Fig. 8 3D-AFM images of the test hair strands (image size 50 μm \times 50 μm)

Fig. 9 Roughness curve of the control hair as analyzed by AFM

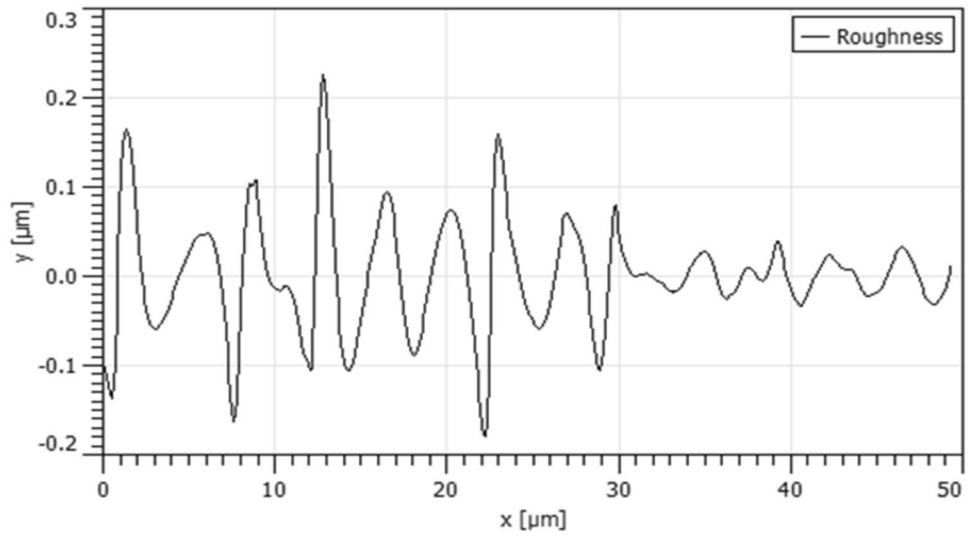
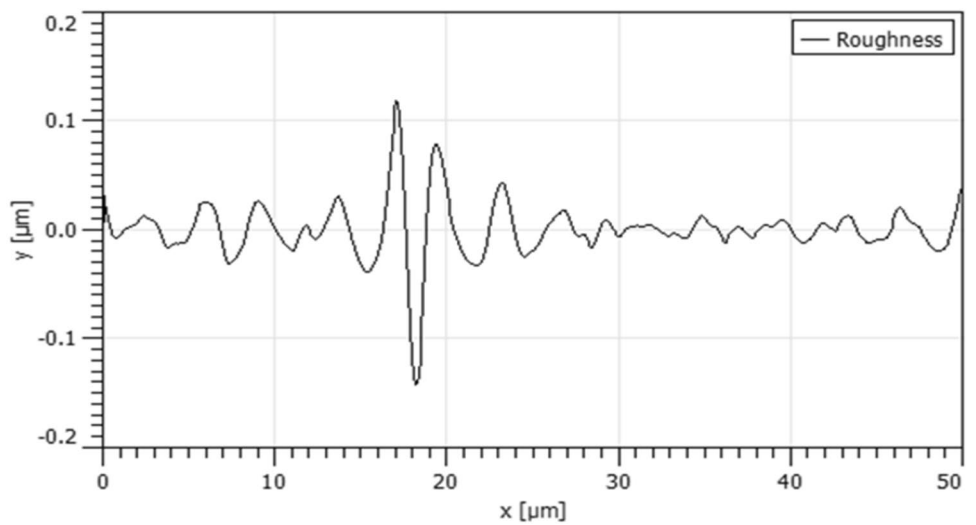


Fig. 10 Roughness curve of the test hair as analyzed by AFM



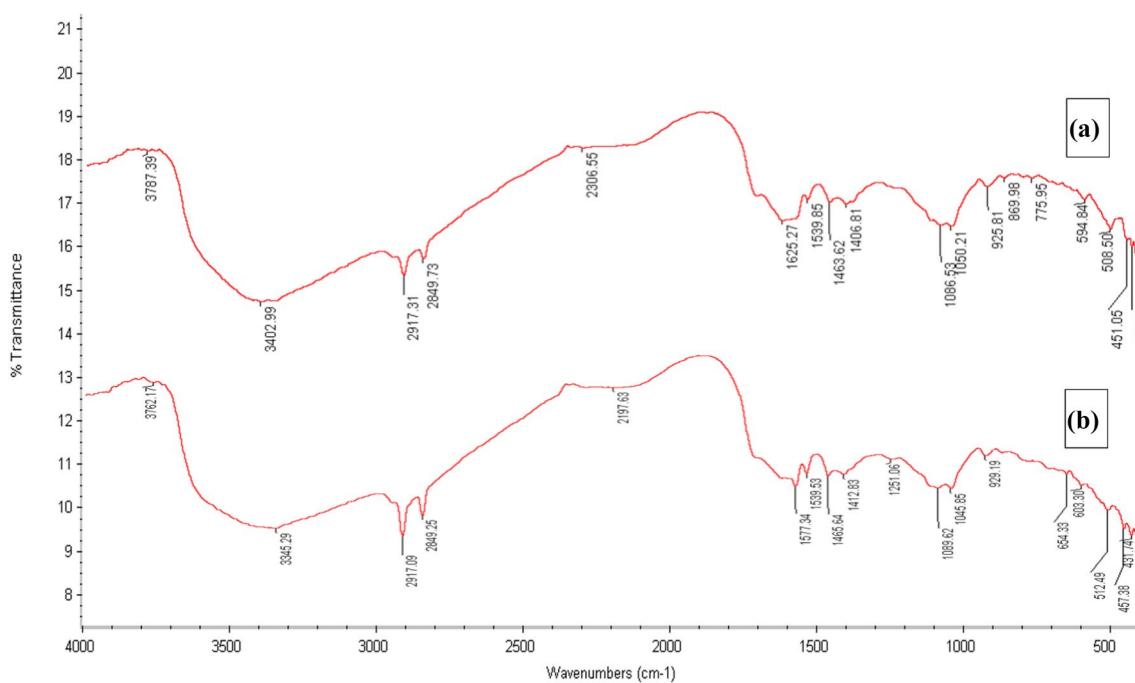


Fig. 11 FTIR spectrum of a. control hair strands and b. test hair strands

Table 2 FTIR spectrum band assignment of the control hair strands

Band	Intensity	Identified functional group
411	13.785	-
435.90	14.777	S-S disulfide bond
451.05	14.960	-
508.50	15.303	-
594.84	16.242	-
775.95	16.899	-
869.98	16.922	-
925.81	16.651	-
1050.21	15.404	C-N Amine
1036.53	15.437	Cysteine-S-thiosulphate
1406.81	16.106	-CH ₃ Alkane
1463.62	16.160	-CH ₂ Alkane
1539.85	16.257	-
1625.27	15.577	C=O Amide
2306.55	17.861	-
2849.73	14.379	O-H Carboxylic acid
2917.31	13.872	C-H alkane
3402.99	13.028	N-H amine
3787.39	17.759	-

3.5 Fourier transform infrared spectroscopy (FTIR)

Upon the examination of the FTIR spectra of both hair samples (Fig. 11), the highest and broadest band at 3402.99 cm^{-1} and 3345.29 cm^{-1} in the spectra of control and test groups respectively indicates the presence of NH amine group that is the main functional group of amino acids (Table 2).

Table 3 FTIR spectrum band assignment of the test hair strands

Band	Intensity	Identified functional group
409.01	7.520	–
431.74	7.623	S–S disulfide bond
457.38	7.799	–
512.49	8.297	–
603.30	8.939	–
654.33	9.244	–
929.19	9.734	–
1045.85	8.865	Cysteine-S-thiosulphate
1089.62	8.886	C–N Amine
1251.06	9.633	Amide III
1412.83	9.238	–CH ₃ Alkane
1465.64	9.201	–CH ₂ Alkane
1539.53	9.258	–
1577.34	8.921	C=C aromatic
2197.63	11.522	–
2849.25	8.162	O–H Carboxylic acid
2917.09	7.613	C–H alkane
3345.29	7.817	N–H amine
3762.17	11.652	–

As shown in Tables 2 and 3, the S–S disulfide bond appears at 431.74 cm⁻¹ in the test group, while it appears 435.90 cm⁻¹ in the control group. Another band at 2849.25 cm⁻¹ was clearly more sharp in the test group than the control, it is identified as the OH group of carboxylic acid that indicates the presence of phenylalanine. Cysteine-S-thiosulphate bands appear at 1036.53 cm⁻¹ and 1045.85 cm⁻¹ in the spectra of control and test groups respectively which is the amino acid responsible for the building of disulfide bonds that adds rigidity and strength to the hair structure. Along with CH₂ alkane and CH₃ alkane bands that appear at 1465.64 cm⁻¹ and 1412.83 cm⁻¹ respectively in the spectra of test group and at 1463.62 cm⁻¹ and 1406.81 cm⁻¹ respectively in the spectra of control group both of them are essential functional groups found mostly in all of the amino acids structures. Nevertheless, there is no significant difference between the amino acids found in the test group and those found in the control group.

3.6 Raman spectroscopy

As depicted in Table 4 and Figs. 12 and 13, the control group has a small peak at 501 cm⁻¹ for S–S stretching vibrations of the disulfide bond that are essential for stabilization of keratin giving the hair its physical and mechanical strength. Moreover, control group has only two peaks at 586.72 cm⁻¹ and 704.88 cm⁻¹ C–S bonds which are residues for cysteine amino acids essential also for keratin structure. While in test group, there was a small broad peak detected at 423 cm⁻¹ for the S–S but stronger peaks than the control group for the C–S aliphatic at 659.34 cm⁻¹ and 791.13 cm⁻¹ indicating tighter bonds and a C–S aromatic ring chain was detected at 1092.91 cm⁻¹ proving stronger C–S bonds. The phenylalanine peak at 1026 cm⁻¹ is a sharper single peak in control group, while, in the test group it is a bigger doublet peak at 1002 cm⁻¹. This might indicate that in the control group, there are holes in the cuticle layer (as observed also in the SEM images) due to phenylalanine presence is more abundant in cortex layer than cuticle. Control has more tyrosine which is also present in the cortex layer more than the cuticle layer and functional group S–H indicating the presence of either methionine or cysteine with less C–H stretching bonds at the end of the spectrum. On the other hand, test group has more tryptophan, S–H group for methionine or cysteine and histidine. The control group has one amide III α -helix and amide I unordered and amide I β -sheet, while the test group contains one amide III β -sheet and two amide I β -sheet. Amide conformation indicates a change in the structural and mechanical properties of keratin, thus, the presence of more β -sheet conformation in the test group might indicate a smoothing effect. Amide III α -helix is also present in cortex layer more than cuticle layer, so it was not detected at test hair group and the presence of amide unordered might indicate formation of random coils due to damage.

Table 4 Confocal raman spectrum band assignment of control and test hair strands

Control		Test	
Band	Identification	Band	Identification
500.02	S-S	423	υS-S
586.76	C-S aliphatic	659.34	υC-S aliphatic
704.88	C-S aliphatic	791.13	υC-S aliphatic
810.64	Tyrosine		
911	υ(C-O-C)	814	υ(C-O-C)
1026	υ(CC) aromatic ring chain Phenylalanine	897.91	υ(O-O) Tryptophan
1137.23	Tyrosine	1001.50	Phenylalanine
1258.3	Amide III α-helix	1092.91	υ(C-S) aromatic ring chain
1503.15	CC aromatic ring tryptophan	1224.19	Amide III β-sheet
1645.99	Amide I unordered	1347.76	υ(C-(NO ₂)) Tryptophan
1682.5	Amide I β-sheet	1495.44	υCC aromatic ring
2510.58	υS-H	1630	Amide I β-sheet Parallel
2524.24	Methionine, Cystine		
2595.27			
2875.26	υC-H	1683.51	Amide I β-sheet Antiparallel
3040	υ=C-H	1797.46- 1900	υC=C
		2563.9	υS-H
		2587.99	
		2827.99	υC-H
		2967.70	
		3000.51	
		3140.26	υO-H Histidine

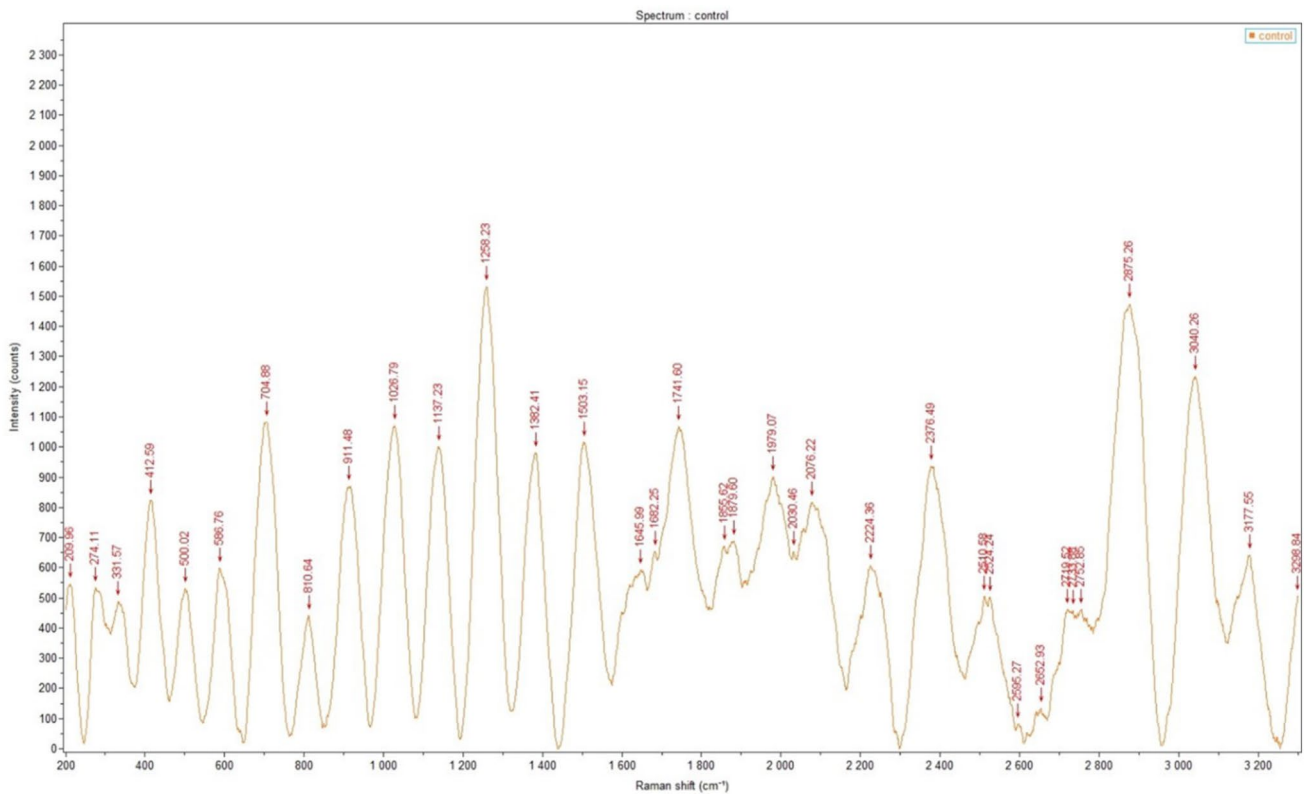


Fig. 12 Confocal raman spectrum of control hair strands

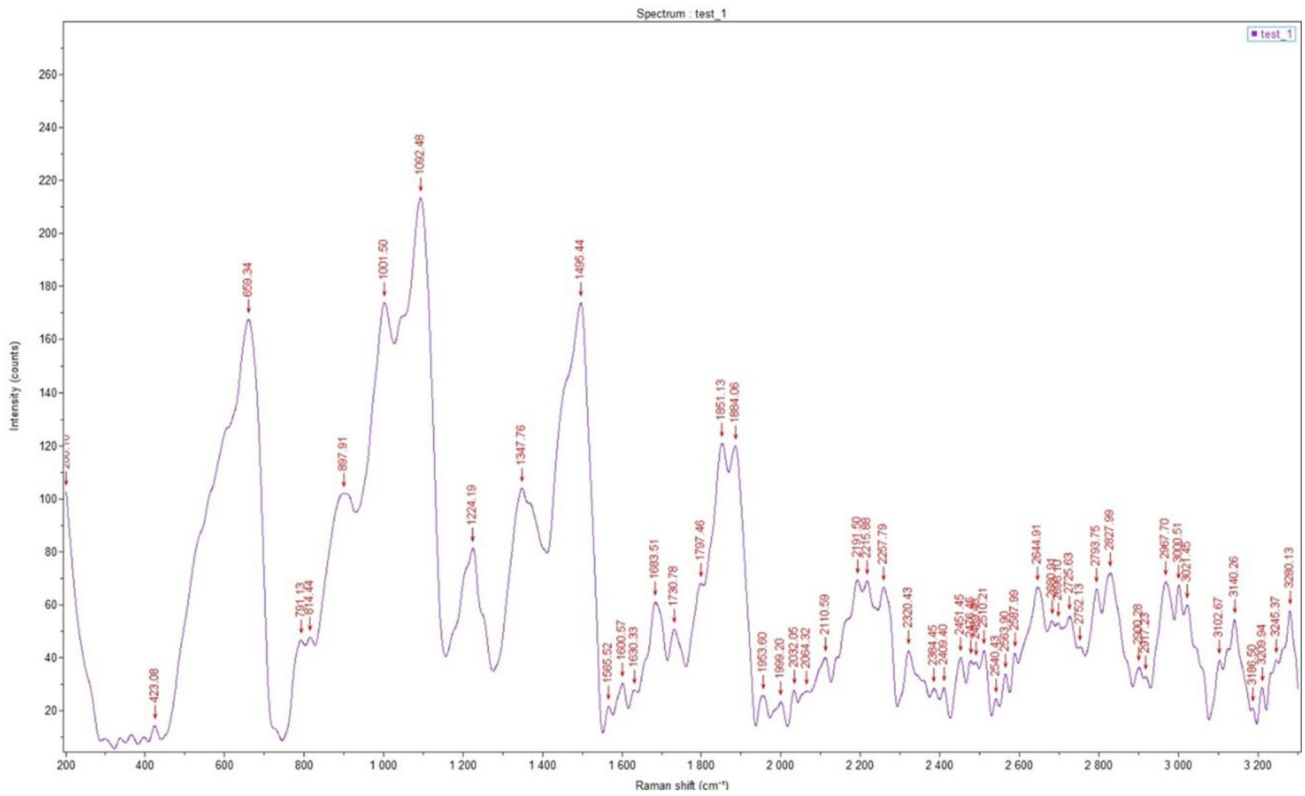


Fig. 13 Confocal raman spectrum of test hair strands

Fig. 14 DSC curve of the control hair sample

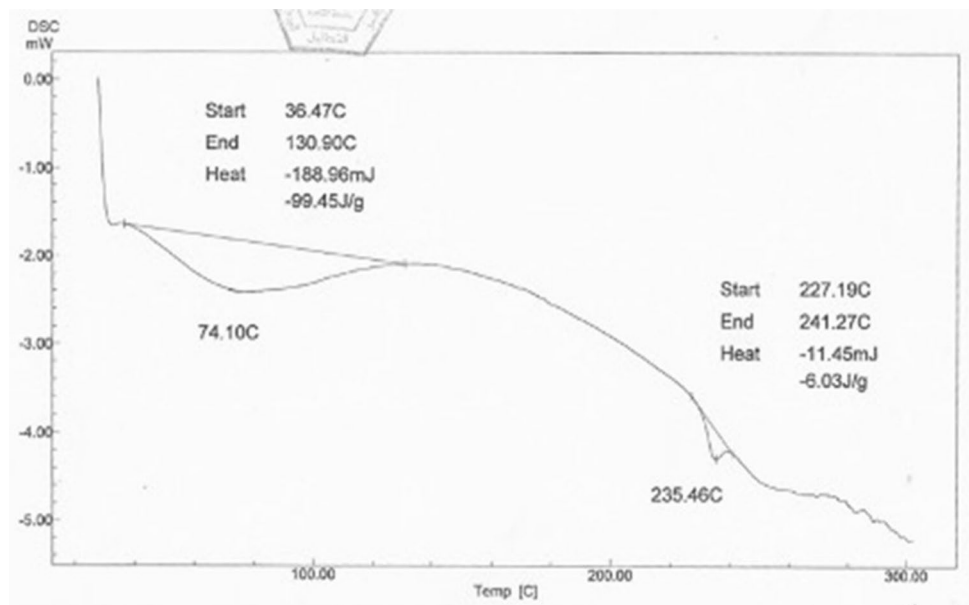


Fig. 15 DSC curve of the test hair sample

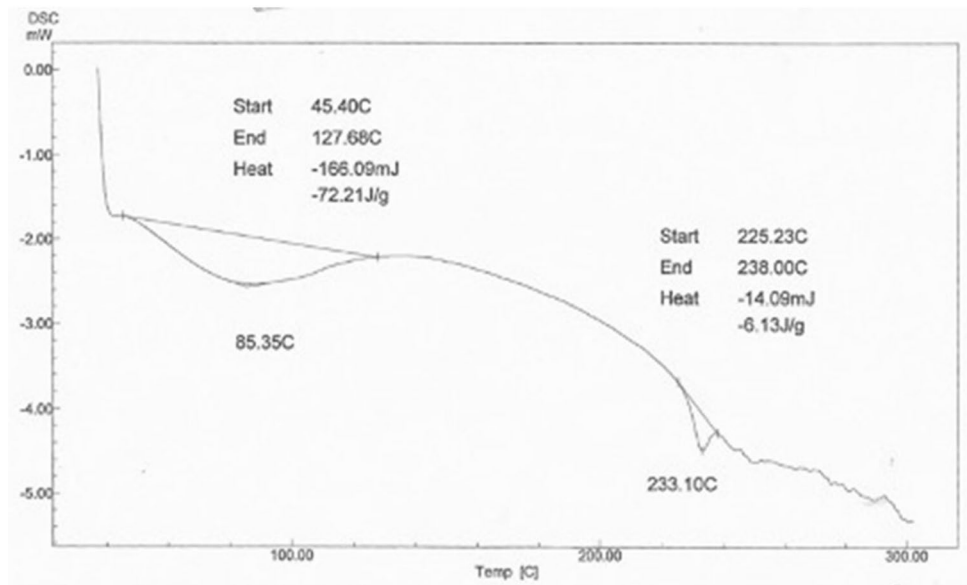


Table 5 TGA analysis of control and test hair groups

	Control hair group	Test hair group
Original weight of each sample	6.069 mg	2.860 mg
Start and end temperatures in 1st peak and weight loss	32.03 to 164.28 °C Weight loss: - 0.735 mg (- 12.111%)	38.22 to 155.77 °C Weight loss: - 0.250 mg (- 8.741%)
Start and end temperatures in 2nd peak and weight loss	164.28 to 301.73 °C Weight loss: - 1.81 mg (- 29.824%)	155.77 to 301.32 °C Weight loss: - 0.861 mg (- 30.105%)
Total weight loss	- 2.543 mg (- 49.901%)	- 1.111 mg (- 38.846%)

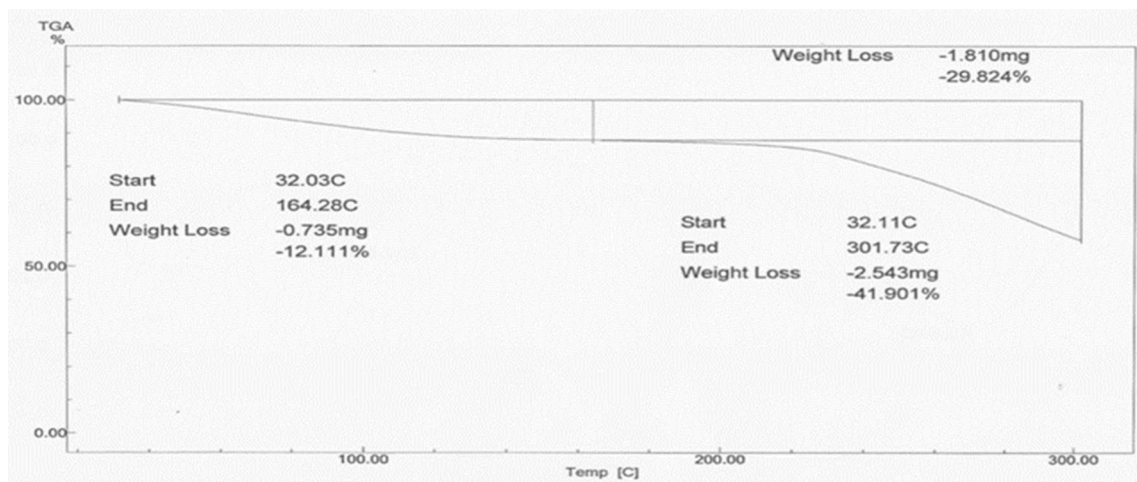


Fig. 16 TGA curve of the control hair group

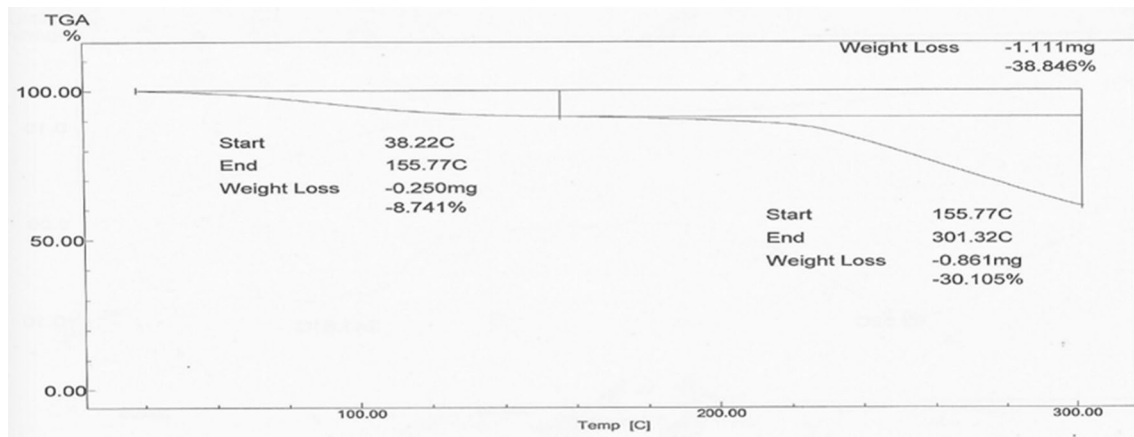


Fig. 17 TGA curve of the test hair group

3.7 Differential scanning calorimetry (DSC)

The DSC curves of the hair strands showed 2 major endothermic peaks. As shown in Figs. 14 And 15, the test sample showed variation in the first endothermic peak – 72.21 J/g compared to the control group – 99.45 J/g while the second peak showed a negligible difference – 6.03 and – 6.13 J/g for the control and test groups respectively. The first endothermic peak can be assigned to the loss of water content while the second endothermic peak can be due to the denaturation of the keratin fibers.

3.8 Thermogravimetric analysis (TGA)

From observing the two TGA curves of both hair groups (Table 5, Figs. 16, 17), it is noticeable that the weight loss in the control group during the first heating interval 32.03 to 164.28 °C was – 0.735 mg, representing – 12.111% of the total weight, while that of the test group at 38.22 to 155.77 °C was – 0.250 mg representing – 8.741%. Similar results were found during the second heating interval, as the control group lost – 1.81 mg representing – 29.824% upon raising the temperature from 164.28 to 301.73 °C while, the test group lost – 0.861 mg representing – 30.105% upon raising the temperature from 155.77 to 301.32 °C. Thus, the total weight loss was – 2.543 mg representing – 49.901% and – 1.111 mg representing – 38.846% for the control and test groups respectively.

4 Discussion

After extracting the protein from *S.platensis*, it was subjected to amino acid analysis, where histidine was found to be the most abundant amino acid and it was reported by Oshimura et al. [17] that histidine and phenyl alanine can improve the hair tensile strength and alanine increases the hair surface hydrophobicity especially in bleached hair. Other identified amino acids include arginine, which contains a guanidinium group that has a high affinity for hair protein and thus arginine is used as a base or anchor to help other materials with weak affinity to hair attach to the hair particle and impart their effect. Moreover, glutamine have high isoionic value, so it binds strongly to hair keratin and thus increases the contact time of the formulation and the hair particle [18]. According to Bayer et al. [19], leucine and valine also enhance the general protein synthesis as they are a building block of the potassium channels which are membrane proteins in the hair strands and follicles so supporting the hair with those amino acids increase the potassium channel function and this leads to the hair function improvement.

The results of *S.platensis* protein analysis comes in accordance with the results of [5, 20, 21], however, they reported that the most abundant amino acid was glutamic acid. This difference could be attributed to the variation of the cultivation period in relation to the sunlight period, the climate, the cultivation environment, the cultivation technique and the soil enrichment by fertilizers.

To evaluate the effects of the protein extract on natural human hair, two cream formulations (test and control) were prepared adopting the method of [12]. Both creams were applied to the hair strands groups and immediate visual results were seen as the hair in the test group was more manageable, smoother and took a shorter duration of brushing than control group as well as an increase in shine and a reduction in split ends. For more reliable data, SEM was used as it is a powerful tool in indicating early hair damage compared to other methods like dermoscopy, normal clinical examination and hair microscopy [22]. The morphological variations between the control and the test groups were clear under the SEM, where the control hair group was more damaged with the presence of cracks and holes on the cuticle surface as well as a higher level of roughness on the contrary from the test hair group. Likewise, the ultra-structures and morphological changes of the hair fibers were viewed by TEM that showed an obvious difference between the inner structure of the test hair group and that of the control hair group. Botchkarev and Paus [23] used TEM to provide a technical guide to study the hair structure, showing a number of photomicrographs that reveals the hair cortex and cuticle and they reported that keratin bundles occur randomly in the cytoplasm of hair Cx cells, which was observed in the cross section. However, the hair cuticle is a single layer although it may appear as multiple layers in the cross section, keratinization of the cuticle proceeds with accumulation of highly electron dense granules found at the distal side of the cytoplasm, and in damaged hair cases there will be no areas of keratinization, and it will appear as a stone wall construction which is called the exocuticle. Our results show a highly porous exocuticle and an intact cortex with numerous keratin content in the control group but not the test group which supports the beneficial effects of *S. platensis* proteins on hair.

Furthermore, both hair samples were analyzed using AFM and a remarkable difference between the test hair sample and control hair sample was observed. AFM enables the evaluation of hair surface beyond the scope of optical and electronic microscopy, where the images are generated by measuring the forces of attraction or repulsion between the hair surface and a very fine probe which is used to scan the hair samples. Thus, AFM provides a very good tool to evaluate certain hair strain properties, such as structural and morphological variations [16]. Basically, it is advantageous to use hair treatments to protect the cuticle from the hydration to reduce the step height and the hair damage susceptibility which means that the hair weakens when the step height increases [24], the test cream reduced the space between the steps from 336.5 to 259.6 nm and the roughness average reduced from 43.5 to 16 nm which reflects its potential to reduce the hair damage.

To evaluate the physical properties of the hair strands and the moisturizing action of the cream, in addition to, the structural changes on hair, FTIR was used. The bands of the functional groups of the amino acids and the structure of hair keratin were identified, but there was no significant difference between the test and control hair groups. Consequently, the confocal raman spectroscopy analysis was done, where, the wavenumber range of $1750\text{--}450\text{ cm}^{-1}$ is more indicative of the changes in the keratin fibers due to the rich vibrational information. The spectra results show major differences specifically in this range between the control and test hair group. Essendoubi et al. [25] focused on the S–S stretching band of the disulfide bonds and the keratin conformations amide 1 α -helix and β -sheet which are essential for the structural properties of keratin, indicating that when β -sheet conformation is more present this is related to increased hair smoothness. In our study, the test hair group showed three bands with β -sheet conformation while the control group showed only one β -sheet, one α -helix and one underordered conformation indicating that *S. platensis* proteins might have a smoothing effect on reducing the hair frizz. This could be attributed to the interaction between the proteins and the keratin in the stratum corneum promoting the conversion of a helix to β -sheet resulting in no frizz effect. It was also observed that the test group showed much lower levels of S–S disulfide bands than the control group, this may be due to the action of *S. platensis* proteins on the keratin tertiary structure by lowering the intensity of the disulfide bond in cysteine which adds to the perceived smoothing effect. In addition, the test group contains higher amount of amide β -sheet conformation bands while the control group contains one amide α -helix, one β -sheet and one unordered. This relates to the SEM results in which the control group had multiple cracks and holes uncovering the cortex layer beneath thus the known indicators of the cortex layer were shown more on the spectra and the test group which had a fully intact cuticle layer showed more indicators of the cuticle layer.

Concerning the thermal analysis of the hair strands, a combination of DSC and TGA was employed to characterize the samples, where, the DSC assess the mass variation, while the TGA is used to assess the mass loss [16]. The DSC thermograms analysis showed an endothermic peak at around 225 °C in the test group. There is a possibility that the higher temperatures of keratin denaturation in our experiment is due to the cream constituents which preserved the moisture content more than the control group and therefore the keratin fibers were able to withstand higher temperatures before denaturation. Besides, the first endothermic peak in the test group thermogram showed higher temperature compared to the control group as it required more energy to release the water which can be due to the better maintaining of moisture content in the test group. According to Belletti et al. [26], these results imply that either the treatment increased the water

content in the hair fiber and so the amount energy to release the water content would be higher or the hindrance of the water release by the treatment due to formation of a barrier that prevented the easy release. Moreover, the TGA analysis clearly showed that the treatment with *S. platensis* proteins was effective on the thermal stability of the hair which was evident by the variability in weight loss between the test and control groups where the control group lost more weight (– 49.901%) compared to the test group (– 38.846%). According to Dankers [27], the initial mass loss is attributed to the loss of water whether loosely bound or tightly bound water. Our results showed a difference in the initial weight loss between the test group (– 8.741%) and the control group (– 12.111%). On the other hand, the second weight loss is basically due to the denaturation of the hair keratin and organic degradation, in our results the difference in the second weight loss is negligible, the test group was (– 30.105%) and the control group (– 29.824%) while the difference in the second weight loss. These findings support that the *S. platensis* protein was effective in preventing the loss of moisture content of the hair and maintaining its humidity.

S. platensis proves to be a great protein source that could be incorporated in hair care formulations with multiple advantages over the traditional protein sources used in the market. Overcoming the drawbacks of animal protein sources which are unethically extracted as well as environmentally damaging and plant protein sources containing insufficient amino acids unlike *S. platensis* as proven by the amino acid analysis. *S. platensis* protein has the potential to become a remarkably effective active ingredient by offering a smoothing effect, reducing frizz, strengthening the hair particle, and offering a better overall appearance.

Author contributions Omar A. Elkady and Mahitab H. El Bishbishy contributed to the study conception and design. Material preparation, data collection and analysis were performed by Mahitab H. El Bishbishy and Islam M. Mannaa. The first draft of the manuscript was written by Omar A. Elkady and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

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Data availability The datasets generated or analyzed during the current study are available from the corresponding author on reasonable request.

Declarations

Ethics approval and consent to participate This study is a model to test a new hair formulation containing *S. platensis* protein on donated hair strands and it involves using the minimal amount of this human biological material to reach significant results.

Competing interests The authors have no relevant financial or non-financial interests to disclose.

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