

Targeted keratin repair: A plant-based approach to hair strength and surface restoration

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Abstract

Objective: Chemical and thermal stress disrupts the intramolecular and intermolecular interactions that stabilize hair keratin, leading to structural degradation and reduced mechanical strength. This study evaluates a plant-derived amino acid complex (*Avena strigosa* seed extract) for its ability to reinforce keratin intra-bonding and restore hair fibre integrity.

Methods: Chemically damaged human hair was treated with formulations containing *A. strigosa* complex. Structural and surface changes were assessed by scanning electron microscopy (SEM), inverse gas chromatography (IGC) and glossmetry. Mechanical performance was quantified via tensile testing. Molecular interaction with keratin was examined using nano-differential scanning fluorimetry (nano-DSF) and thiol-binding fluorescence assays. A split-head in vivo study ($n = 20$) evaluated sensory and visual attributes after a single shampoo application.

Results: Treatment with *A. strigosa* complex significantly improved hair properties. SEM revealed cuticle resealing and reduced porosity; Brunauer–Emmett–Teller-specific surface area decreased by 23% compared to bleached control. Gloss increased by 105% after five wash cycles, and tensile strength improved by up to 30% in leave-on treatments ($p < 0.001$). Nano-DSF indicated direct interaction with keratin (thermal shift: -3.18°C), and thiol-binding assays showed a 9.2% reduction in free sulfhydryl groups, confirming protection of disulphide bonds. In vivo, hair treated with *A. strigosa* complex scored higher for shine and smoothness versus placebo (mean difference + 1 point; $p < 0.05$).

Conclusion: *A. strigosa* complex reinforces keratin intra-bonding by stabilizing hydrogen and ionic interactions and preserving disulphide integrity, resulting in measurable improvements in strength, gloss and surface quality. This targeted approach offers a validated strategy for repairing chemically damaged hair.

KEYWORDS

chemical analysis, claim substantiation, hair biomechanics, hair fibre integrity, hair treatment, keratin intra-bonding, thermal shift assay

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Résumé

Objectif: Les contraintes chimiques et thermiques perturbent les interactions intramoléculaires et intermoléculaires qui stabilisent la kératine capillaire, entraînant une dégradation structurelle et une diminution de la résistance mécanique. Cette étude évalue un complexe d'acides aminés d'origine végétale (extrait de graines d'*Avena strigosa*) pour sa capacité à renforcer les liaisons internes de la kératine et à restaurer l'intégrité des fibres capillaires.

Méthodes: Des cheveux humains chimiquement endommagés ont été traités avec des formulations contenant le complexe *A. strigosa*. Les changements structurels et de surface ont été évalués par microscopie électronique à balayage (MEB), chromatographie en phase gazeuse inverse (CGI) et glossométrie. Les performances mécaniques ont été quantifiées par des essais de traction. L'interaction moléculaire avec la kératine a été examinée à l'aide d'une fluorimétrie à balayage nanodifférentielle (nano-DSF) et d'essais de fluorescence de liaison aux thiols. Une étude *in vivo* à tête divisée ($n=20$) a évalué les attributs sensoriels et visuels après une seule application de shampooing.

Résultats: Le traitement par le complexe *A. strigosa* a significativement amélioré les propriétés des cheveux. La MEB a révélé un rescallage des cuticules et une porosité réduite; la surface spécifique BET a diminué de 23% par rapport au témoin décoloré. La brillance a augmenté de 105% après cinq cycles de lavage, et la résistance à la traction s'est améliorée jusqu'à 30% dans les soins sans rinçage ($p < 0.001$). Le nano-DSF indiquait une interaction directe avec la kératine (déplacement thermique: $-3,18^{\circ}\text{C}$), et les tests de liaison au thiol ont montré une réduction de 9,2% des groupes sulfhydryles libres, confirmant la protection des liaisons disulfure. *In vivo*, les cheveux traités par le complexe *A. strigosa* ont obtenu un score plus élevé pour la brillance et la douceur par rapport au placebo (différence moyenne +1 point; $p < 0.05$).

Conclusion: Le complexe *A. strigosa* renforce les liaisons internes de la kératine en stabilisant les interactions hydrogène et ioniques et en préservant l'intégrité des ponts disulfure, ce qui entraîne des améliorations mesurables de la résistance, de la brillance et de la qualité de surface. Cette approche ciblée offre une stratégie validée pour réparer les cheveux endommagés chimiquement.

INTRODUCTION

Human hair is a keratin-based composite whose mechanical performance depends on a hierarchical protein architecture. At the molecular level, keratin filaments and the surrounding matrix are stabilized by a dense network of intramolecular and intermolecular interactions, including covalent disulphide crosslinks as well as hydrogen, ionic and hydrophobic interactions (Figure 1). The chemistry and distribution of amino acid side chains govern the formation and stability of these interactions, which together maintain fibre cohesion, elasticity and surface quality under everyday stress.

In hair, the most influential amino acids include cysteine, glutamic acid, serine, arginine, threonine, lysine, aspartic acid and various hydrophobic residues such as leucine, isoleucine and phenylalanine (Table 1) [1–6].

Keratin architecture is governed by amino acid chemistry and the interactions they enable. Disulphide bonds, the most robust and permanent links, form exclusively between cysteine residues, creating cystine bridges. Their abundance confers mechanical strength and defines natural shape. Chemical treatments (bleaching, perming and relaxing) or intense heat can cleave these bonds; subsequent oxidation to cysteic acid is irreversible and critically weakens the fibre [7, 8].

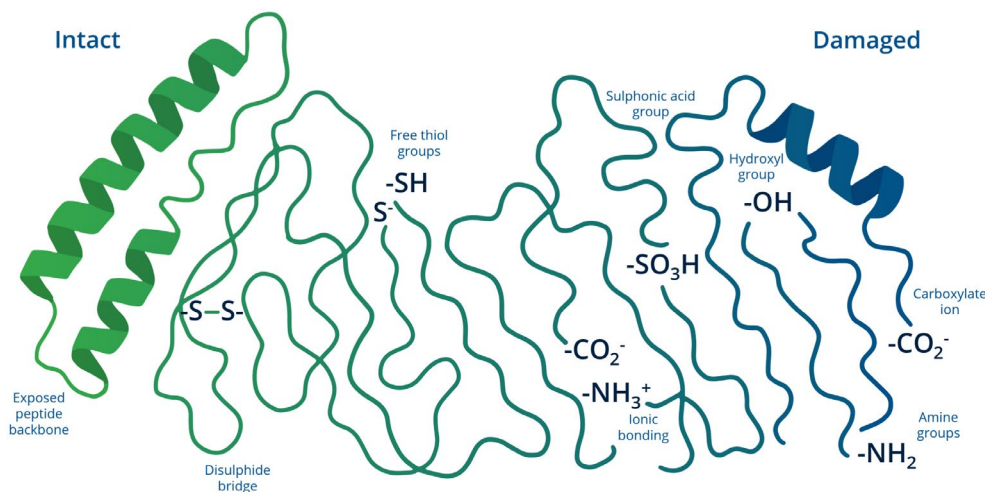


FIGURE 1 Keratin integrity versus damage—what happens inside the hair fibre. Healthy keratin is stabilized by a dense network of disulphide bonds that lock the protein chains together, supported by thiol groups and a well-structured peptide backbone. This architecture gives hair its strength, elasticity and resistance to external stress. When hair is exposed to chemical treatments or oxidative processes, this network begins to break down. Disulphide bonds are cleaved and replaced by sulfonic acid groups, whereas ionic and hydrogen bonding patterns become disrupted. Carboxylate ions and exposed amine groups appear, signalling the collapse of the protein structure. These molecular changes weaken the keratin framework, leaving hair porous, brittle and prone to breakage—a clear illustration of why damaged hair requires targeted repair strategies.

TABLE 1 Most abundant amino acids in human hair shaft.

Amino acid	Approx. % in hair keratin
Cysteine	14–18
Glutamic acid (glutamine)	12–15
Serine	9–11
Arginine	8–9
Threonine	7–9
Leucine	5–7
Valine	5–6
Glycine	5
Aspartic Acid (asparagine)	5
Alanine	4.8

Ionic bonds arise from electrostatic attraction between acidic (glutamic, aspartic) and basic (lysine, arginine) residues, aligning keratin chains and contributing to cohesion. These interactions are highly pH-dependent: protonation under acidic conditions or deprotonation under alkaline conditions disrupts pairings. Although some reform when pH normalizes, repeated chemical exposure progressively depletes charged sites, weakening the network [9].

Hydrogen bonds, formed between polar residues (serine, threonine, tyrosine, asparagine and glutamine) and backbone amides, are individually weak but abundant—approximately nine per disulphide bond—providing

elasticity and responsiveness to water and heat. Wetting breaks many hydrogen bonds, enabling temporary reshaping; drying restores them. Loss of polar residues reduces bonding sites, diminishing flexibility and increasing fatigue [10].

Hydrophobic interactions, driven by non-polar residues (leucine, isoleucine, valine, alanine and phenylalanine), stabilize the cortex and cuticle alignment by clustering away from water. Oxidative or chemical disruption loosens this matrix, increasing porosity, reducing moisture retention and elevating friction, ultimately dulling shine. Collectively, these interactions create the hair shaft's architecture; simultaneous failure, such as in bleaching or thermal stress, can cause extensive, largely irreversible degradation. Preserving hair integrity therefore depends on maintaining these amino acid-driven interactions [9, 11].

Macroscopically, damage manifests as reduced tensile strength, elasticity, gloss and increased friction and split ends [12, 13]. Despite numerous cosmetic repair strategies, most evidence remains phenomenological and rarely demonstrates restoration of keratin bonding in situ under realistic conditions. This gap motivates approaches targeting molecular cohesion and linking mechanism to function. Protein hydrolysates, amino acids and peptide-based actives are widely explored, yet robust substantiation requires convergent readouts connecting keratin stabilization to measurable improvements in structure, surface and mechanics. A multi-modal framework should integrate microscopy for cuticle integrity and porosity,

surface-sensitive methods for energy and roughness, molecular assays for keratin interaction and standardized biomechanical testing, complemented by in-vivo assessments.

Here, we investigate a targeted amino acid complex derived from *Avena strigosa* [14], designed to reinforce intramolecular cohesion within hair keratin by stabilizing hydrogen and ionic interactions while supporting the integrity of existing disulphide bonds. In this context, *keratin intra-bonding* refers to the strengthening of non-covalent interactions and the protection of disulphide bonds, rather than the reformation of covalent disulphide crosslinks.

MATERIALS AND METHODS

Preparation of *Avena strigosa* complex

Avena strigosa (black oat) is valued for its agronomic benefits, including allelopathic activity and nematocidal effects. Despite these properties, the species faced near extinction in Europe by the late twentieth century [14]. The *A. strigosa* complex, obtained via gentle aqueous extraction and vacuum drying, is solubilized in glycerine. Preservation is achieved with pentylene glycol and pH adjustment with citric acid. Lecithin is incorporated for its film-forming properties, reducing friction of hair surface. The finished complex formulation contains the following ingredients (INCI): glycerine, water, pentylene glycol, *Avena strigosa* seed extract, lecithin and citric acid. Experiments in this publication have been performed either with pure *A. strigosa* powder solubilized in water (called *A. strigosa* extract) or using *A. strigosa* complex (including preservation and lecithin) diluted in water.

Ex vivo studies

Molecular interaction studies

To quantify amino acid exchange between damaged hair and an *A. strigosa* (black oat) extract by measuring uptake into hair and release as a marker of daily amino acid loss. Double-bleached human hair (type I) was immersed in a 3% aqueous *A. strigosa* extract for 1 h at room temperature. A control extract solution without hair served as reference. Post incubation, solutions were analysed by UPLC-MS for free amino acids and covalently bound amino acids. Free amino acids were quantified after precipitation; covalently bound amino acids after acid hydrolysis. Chromatographic separation employed gradient

elution with formic acid in water/acetonitrile, and detection used positive-mode mass spectrometry with selected ion monitoring.

Interaction of increasing concentrations of *A. strigosa* extract (1%–10% in 0.5 steps) with recombinant human hair keratin KRT85 (3 μ M) was analysed using nano-differential scanning fluorimetry thermal shift assay (nano-DSF-TSA), which monitors protein unfolding via intrinsic tryptophan and tyrosine fluorescence. Measurements were performed in a 20 mM HEPES pH 8.0, 500 mM NaCl buffer over 20°C–95°C with a heating rate of 1°C·min⁻¹ in a Prometheus Panta instrument (NanoTemper Technologies GmbH). Intrinsic protein fluorescence was excited at 280 nm, and emission was recorded at 330 nm and 350 nm during thermal ramping. Data were analysed with PR.Panta Analysis software from NanoTemper. Protein unfolding was evaluated by calculating the first derivative of the fluorescence signal at 350 nm with respect to temperature. The inflection point (IP) of the resulting transition curve was used to determine the apparent melting temperature (T_m) for the protein. Measurements were done in duplicates. Thermal shift assays (TSA) were applied to detect ligand-induced changes in protein stability, with shifts in T_m indicating stabilizing or destabilizing interactions upon addition of *A. strigosa*.

Reduction of free sulfhydryl groups (–SH) in chemically damaged hair was analysed as follows: Double-bleached human hair tresses (type I) were treated for 20 min with 1 mL of the test product, which was massaged into the tress using gloved fingers. The treatment formulations contained either *A. strigosa* extract (0.3% and 3%), *A. strigosa* complex (1%), maleimide solution (0.15%) or a finished cosmetic benchmark product [INCI: water, bis-aminopropyl diglycol dimaleate, propylene glycol, cetearyl alcohol, hydrolysed soy protein, ascorbic acid (vitamin C), tocopheryl acetate (vitamin E), retinyl palmitate (vitamin A), aloe barbadensis leaf juice, phytantriol, panthenol, Simmondsia chinensis (Jojoba) seed oil, hydrolysed wheat protein, wheat amino acids, behentrimonium methosulphate, cetyl alcohol, glycerin, stearamidopropyl dimethylamine, hydroxyethyl ethylcellulose, Quaternium-91, cetrimonium methosulphate, cetrimonium chloride, fragrance (Parfum), polyquaternium-37, tetrasodium EDTA, magnesium nitrate, butylphenyl methylpropional, methylchloroisothiazolinone, magnesium chloride, methylisothiazolinone, etidronic acid, potassium sorbate, phenoxyethanol, citric acid, disodium EDTA, sodium benzoate, Prunus amygdalus dulcis (sweet almond) oil]. After rinsing and air-drying, fibres were labelled with rhodamine isothiocyanate (RITC), a fluorescent probe for free –SH groups. Fluorescence intensity was quantified to assess the extent of exposed sulfhydryl groups, with maleimide serving as a positive control for maximal binding.

Physical hair properties studies

Surface characterization was performed using inverse gas chromatography (IGC) with a surface energy analyser to determine the Brunauer–Emmett–Teller-specific surface area (BET SSA) and surface energy of hair fibres. Hair tresses were prepared under four distinct conditions, namely virgin and bleached hair, washed twice with a placebo shampoo (INCI: water, potassium sorbate, sodium benzoate, sodium laureth sulphate, cocamidopropyl betaine, sodium chloride and citric acid). Bleached hair with conditioning, washed twice with placebo shampoo and treated after each wash with a commercial conditioner (INCI: water, stearyl alcohol, silicone Quaternium-26, behentrimonium chloride, cetyl alcohol, Parfum, isopropyl alcohol, benzyl alcohol, sodium benzoate, disodium EDTA, citric acid, polysorbate 20, panthenol, panthenyl ethyl ether, limonene, histidine, sodium hydroxide, sodium hyaluronate, phenoxyethanol, biotin). Bleached hair with active treatment, washed twice with a shampoo containing 0.5% *A. strigosa* complex. Hair samples were cut, packed into stainless-steel columns (30 cm length, 4 mm internal diameter) and pre-conditioned for 2 h at 30°C and 0% RH under helium flow to remove adsorbed contaminants and moisture. Retention times were recorded and converted into net retention volumes, which correlate with the physicochemical properties of the sample. Adsorption data were used to construct sorption isotherms, enabling calculation of BET SSA via the BET equation based on octane adsorption. A series of probe molecules (nitrogen, octane, dichloromethane, ethyl acetate, chloroform, ethanol and acetonitrile) were injected at varying surface coverages to quantify dispersive (non-polar) and acid–base (polar) surface energy components. The dispersive component was calculated using the Schultz method, whereas acid–base contributions were derived from polar probe interactions. These measurements provided total surface energy values and acid–base distribution profiles. Analysis across different surface coverages yielded heterogeneity profiles, offering insights into the energetic distribution and uniformity of hair fibre surfaces following each treatment.

Hair surface morphology was assessed by scanning electron microscopy (SEM) following standard preparation protocols. SEM images were evaluated using a damage scoring system ranging from 0 (intact cuticle) to 5 (severe erosion). The repair effect was calculated by comparing treated samples to untreated control. Three equivalent hair tresses (hair type I, 2 g) were used. Prior to treatment, tresses were subjected to five consecutive washings (shampoo with 0% or 0.2% *A. strigosa* complex) and blow-drying cycles (2 min at 60°C) to induce surface damage.

Hair gloss was quantified using a Glossmeter (Courage + Khazaka). Bleached hair tresses (type I) were washed with shampoo containing 0.5% *A. strigosa* complex. For each wash cycle, 1 mL of shampoo was applied to wet tresses for 3 min, rinsed, towel-dried and blow-dried for 3 min at 30 cm distance. The procedure was repeated after one, three and five cycles. A control tress treated with shampoo without *A. strigosa* complex served as reference.

Tensile strength was measured on single hair fibres (bleached type I tresses, hair shaft diameter 0.07–0.08 mm; minimum 30 hairs per condition) using a Z100 ProLine tabletop testing machine equipped with an XForce P transducer (ZwickRoell, Germany). Test parameters were following forward force 0.002 N, E-modulus velocity 1 mm·min⁻¹ and test speed 50 mm·min⁻¹. Hair tresses were treated under three configurations, namely using a rinse-off shampoo (same INCI as above) and a conditioner [INCI: water (aqua), caprylic/capric triglyceride, cetearyl alcohol, polyglyceryl-3 dicitrate/stearate, glyceryl stearate, xanthan gum, sodium benzoate, citric acid, potassium sorbate, sodium stearyl glutamate] containing either 0% or 0.5% *A. strigosa* complex, each applied for 3 min. The number of shampooing treatments was systematically increased to 1, 3 and 5 cycles in order to elucidate the cumulative effects associated with repeated applications.

A leave-on treatment in an aqueous solution containing 0%, 0.25% or 0.5% *A. strigosa* complex for 30 min. All samples were stored overnight in a climate-controlled room (22°C, 55% RH) and kept in sealed containers during testing to prevent humidity variations.

Virgin type I hair tresses were used to assess split end formation. One tress served as untreated control (baseline), whereas two tresses were washed five times with a shampoo containing either 0% or 0.5% *A. strigosa* complex. After treatment, each tress was subjected to 9000 brush strokes combined with hot air exposure (blow dryer, 60°C, 1 h) to induce split ends. Subsequently, 300 individual hairs per tress were examined microscopically for the presence of split ends.

In vivo study

In vivo experiment was conducted in compliance with Good Laboratory Practice (GLP), Good Clinical Practice (GCP) and the principles outlined in the Declaration of Helsinki. Written informed consent was obtained from all participants prior to study initiation.

A split-head study was conducted with 20 female subjects (aged 35–72 years; hair condition: slight to strong

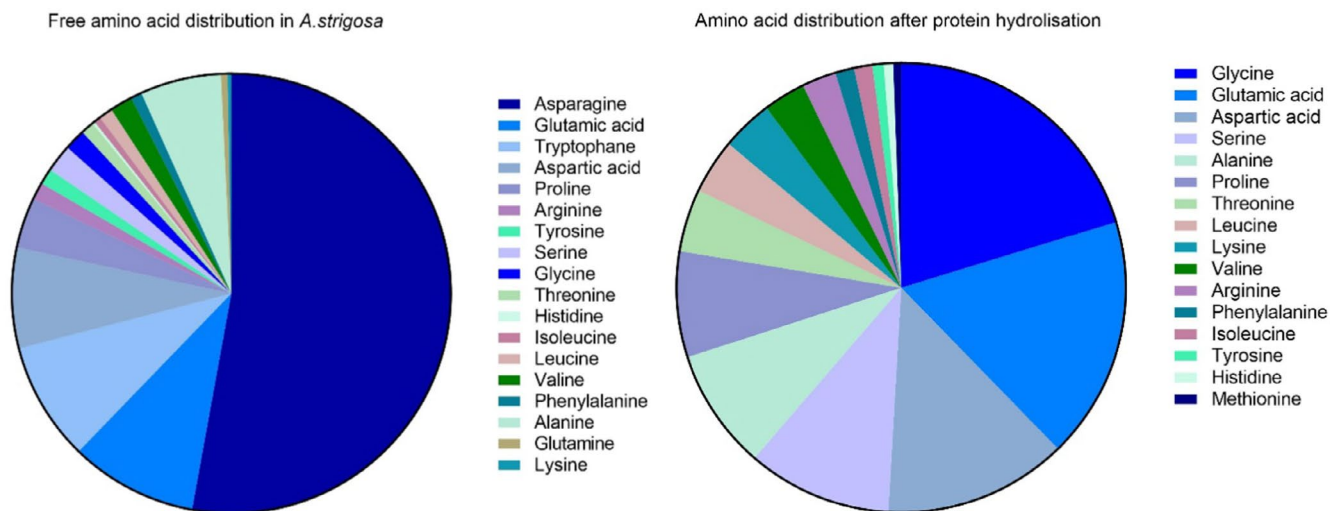


FIGURE 2 Free amino acid content and distribution in *Avena strigosa* extract before and after acid hydrolysis. The relative concentrations of individual amino acids detected in the native extract (left) are compared to the hydrolysed extract (right), as determined by mass spectrometry. Hydrolysis significantly increased the detectable levels of protein-derived amino acids, revealing a broader spectrum and higher overall concentration compared to the non-hydrolysed sample. This indicates that the extract contains both free amino acids and amino acids that become accessible after hydrolysis of peptides and proteins from the extract.

damage; mixed lengths and textures). Two shampoos were compared: a placebo and a test formulation (0% and 0.5% *A. strigosa* complex). Each product was applied as pre-washing (4–6 ml of shampoo) and main washing (2–4 ml of shampoo) to one half of the head. Professional hairdressers assessed application properties during washing (foam texture, foam feel and rinseability) and hairdressing parameters on wet and dry hair (detangling, combing, softness, shine, suppleness, bounce-back and manageability). Ratings were recorded on a 10-point scale (1 = very poor, 10 = excellent).

Statistical analysis

Statistical comparison was done by paired Student's *t*-test for intra-group comparisons and by unpaired Student's *t*-test for inter-group analysis. In case differences between groups were not normally distributed, the Wilcoxon-signed rank test was used. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$. Error bars display standard error of the mean.

RESULTS

The following experiments demonstrate how the amino acids and proteins from *A. strigosa* restore damaged hair structure from within, resulting in a virgin hair-like surface and improved resilience as well as hair feel.

Ex vivo studies

Molecular interaction studies

The initial analysis of *A. strigosa* extract (Figure 2) revealed its amino acid composition before and after hydrolysis, confirming the presence of both free and protein-bound amino acids.

Following immersion of double-bleached hair, mass spectrometry showed a significant increase in free amino acids as well as peptides and protein fragments in the solution compared to the control. Damage to the hair shaft is associated with the leaching of free amino acids such as serine, glycine, threonine, histidine and isoleucine into the surrounding medium, indicating disruption of the proteinaceous matrix. The loss of amino acids, peptides and structural proteins increases porosity and compromises the mechanical integrity of the hair fibre, creating voids within the architecture. These structural gaps necessitate targeted interventions to restore stability. Conversely, free amino acids such as asparagine, glutamic acid and tryptophan were absorbed by bleached hair, suggesting selective incorporation that supports structural repair and fortification. This preferential uptake could be attributed to their chemical features: multiple hydrogen-bond donor and acceptor sites facilitate interactions with polar regions of the damaged matrix, whereas aromatic residues such as tryptophan can engage in hydrophobic pocket stabilization, potentially reinforcing the compromised architecture.

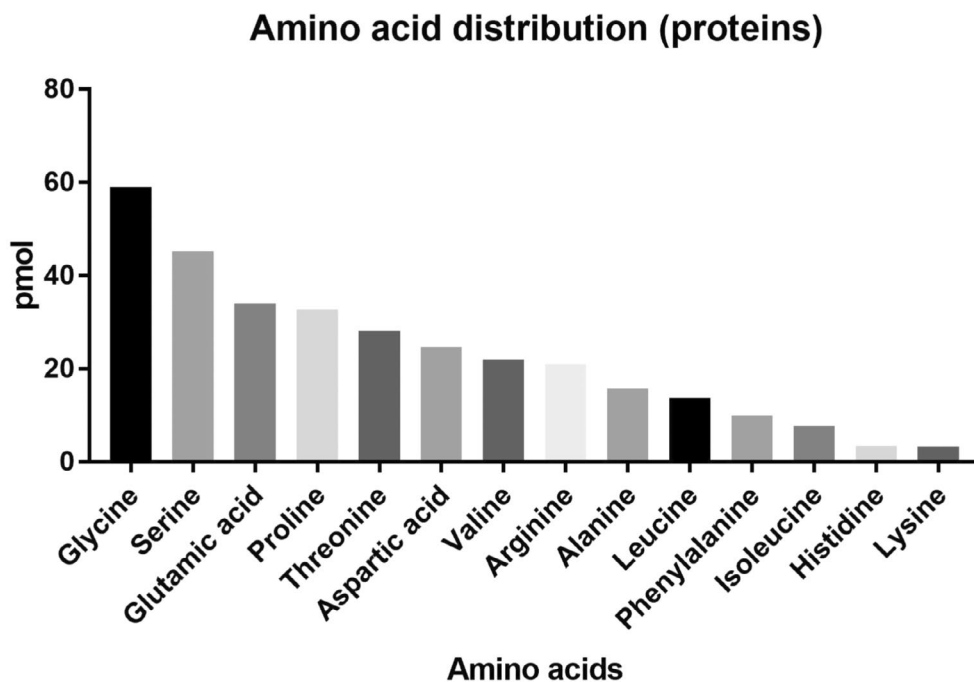


FIGURE 3 Amino acid content and distribution after immersion of damaged hair in *A. strigosa* extract. Hydrolysis of the post-immersion solution markedly increased the detectable levels of protein-derived amino acids, indicating a higher overall concentration compared to the *A. strigosa* extract alone. The graph illustrates the net amino acid content, calculated by subtracting the baseline levels present in the original *A. strigosa* extract.

Hydrolysis of the postimmersion solution further confirmed the presence of substantial protein fragments originating from the hair shaft (Figure 3).

This confirms that severe bleaching causes deep structural damage, resulting in protein-level degradation. With daily structural depletion of keratin in bleached hair, replenishment of these components becomes essential in order to maintain hair integrity. These findings show that *A. strigosa* water extract can serve as a source of essential amino acids for damaged hair, helping to replenish the lost amino acids and contributing to hair repair and resilience. Despite being one of the most abundant amino acids in keratin, cysteine was not detected under the applied analytical conditions and fell below the limit of quantification. This outcome reflects well-documented limitations of acid-hydrolysis-based amino acid analysis, where cysteine readily oxidizes to cystine or further degrades into multiple sulphur-containing species, resulting in unreliable or incomplete detection [15]. Such analytical challenges are particularly prominent in heavily bleached hair, where extensive oxidative modification of sulfur residues is known to occur. Therefore, the absence of a quantifiable cysteine or cystine signal is consistent with the expected chemical consequences of severe bleaching and further supports the conclusion that the fibre experienced substantial protein-level damage. Alternative methodologies such as performic acid

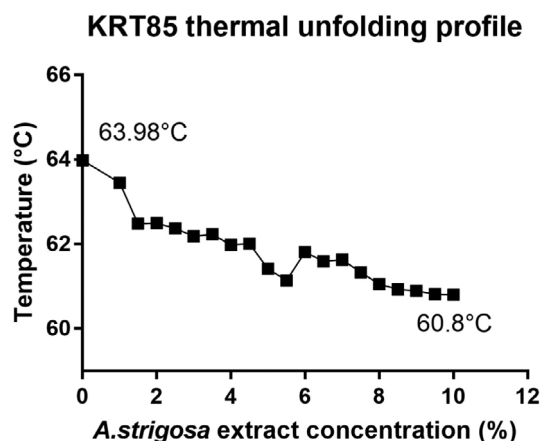


FIGURE 4 *Avena strigosa* decreases the thermal stability of KRT85. Nano-Differential Scanning Fluorimetry (nano-DSF) analysis revealed a dose-dependent decrease in the melting temperature (T_m) of KRT85 upon addition of *A. strigosa* extract. The maximum thermal shift observed was -3.18°C , indicating a direct interaction with the protein structure.

oxidation or alkaline hydrolysis can be employed to enable reliable quantification. The presence of *A. strigosa* extract led to a measurable shift in the melting temperature (T_m) of KRT85, with a maximum decrease of 3.18°C (Figure 4). This experiment suggests that amino acids and peptides exhibit a clear interaction with KRT85. The

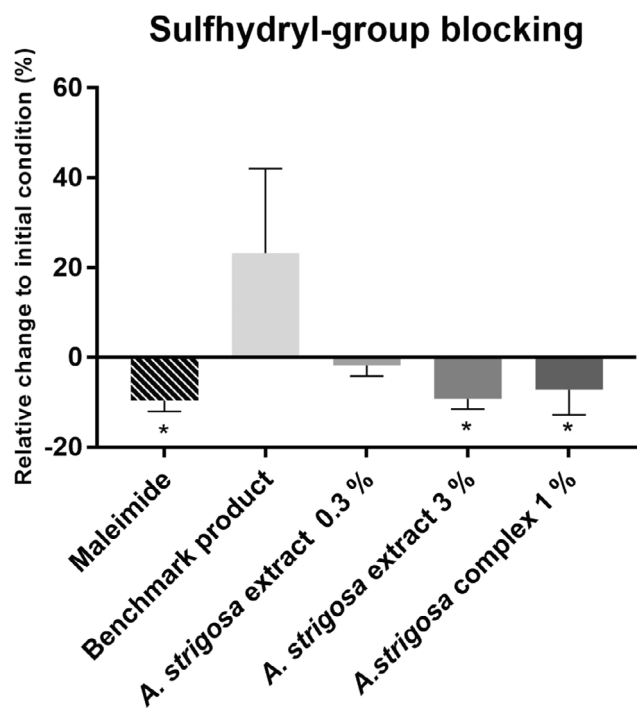


FIGURE 5 *Avena strigosa* extract (3%) and *A. strigosa* complex (1%) reduce free sulfhydryl groups in double-bleached hair. Hair strands were treated with various formulations and labelled with RITC, which binds to exposed -SH groups. Fluorescence quantification revealed a significant reduction in RITC binding, indicating repair or protection of disulphide bonds.

observed decrease in melting temperature (T_m) indicates increased flexibility of KRT85 secondary structures, likely due to the formation of new hydrogen bonds or similar stabilizing interactions. When these findings are considered alongside the selective uptake of amino acids by bleached hair, it appears plausible that, in an aqueous environment, the reduction in T_m reflects these interactions. Under native conditions without excess water, such interactions would be expected to exert a stabilizing effect on the hair fibre architecture, as confirmed by subsequent experimental results.

Chemical damage, such as bleaching, disrupts disulphide bonds and exposes numerous free sulfhydryl (-SH) groups, critically weakening the keratin network. These reactive sites accelerate oxidative degradation, as evidenced by a marked increase in RITC incorporation (higher RITC fluorescence signal = greater damage). Pre-treatment with maleimide sharply reduced fluorescence, validating the assay as a reliable indicator of thiol availability. Among the tested treatments, *A. strigosa* extract (3%) and *A. strigosa* complex (1%) produced the strongest effects, lowering fluorescence by up to 9.2% compared to the bleached control ($p < 0.05$), while 0.3% *A. strigosa* extract showed only minor impact, confirming a dose-dependent response (Figure 5). This reduction in reactive sulfhydryl

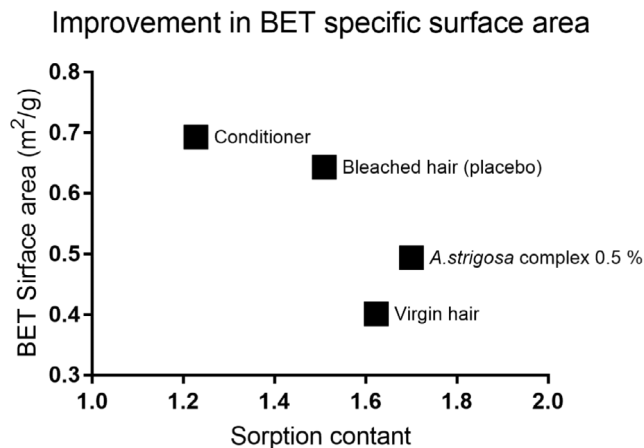


FIGURE 6 *Avena strigosa* complex restores hair surface properties after chemical damage as indicated by the Brunauer–Emmett–Teller-specific surface area (BET SSA) and sorption constant resulting from the IGC analysis of virgin, bleached, placebo-treated and 0.5% *A. strigosa* complex-treated hair fibres. Compared to virgin hair, bleaching increased BET surface area and slightly decreased sorption constant, indicating surface roughness with minor energetic changes. Conditioner treatment further increased surface area and strongly reduced sorption constant, suggesting coating deposition rather than cuticle repair. In contrast, compared to bleached hair alone, treatment with *A. strigosa* complex significantly reduced BET surface area and increased sorption constant towards virgin hair levels, indicating effective restoration of structural integrity and surface energy, thereby improving cuticle recovery and smoothness.

groups indicates protection of existing disulphide bonds and stabilization of the keratin network under stress conditions. These molecular effects translate into measurable functional benefits. Notably, the benchmark product increased fluorescence rather than reducing it.

Physical hair properties studies

Bleaching caused a significant increase in BET SSA from $0.40 m^2 \cdot g^{-1}$ in virgin hair to $0.64 m^2 \cdot g^{-1}$ (Figure 6), indicating a rougher and more porous surface and confirming substantial cuticle disruption. Treatment with a conventional conditioner did not repair the surface; instead, it further increased BET SSA to $0.69 m^2 \cdot g^{-1}$ (making the surface even rougher) and reduced the sorption constant, indicating deposition of a coating that masks damage rather than restoring structural integrity. In contrast, treatment with *A. strigosa* complex reversed these effects: at 0.5%, BET SSA decreased to $0.49 m^2 \cdot g^{-1}$, approaching virgin hair levels (indicating smoother cuticles), while the sorption constant increased, reflecting improved surface uniformity and better physicochemical properties.

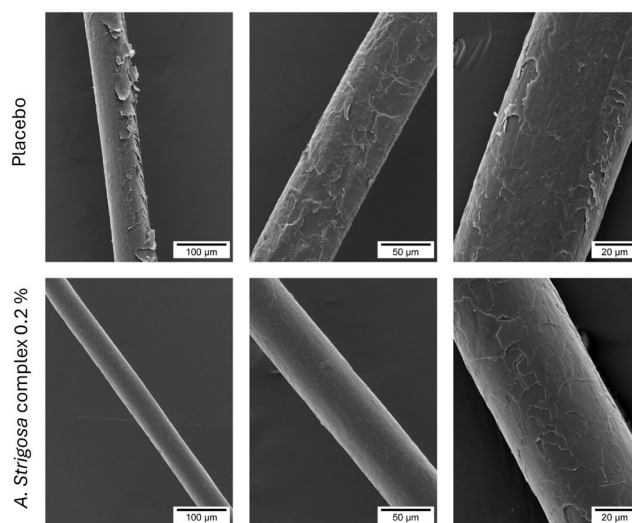
Surface energy analysis showed that bleaching significantly increased total surface energy heterogeneity ($\Delta = 26.7 \text{ mJ}\cdot\text{m}^{-2}$) compared to virgin hair ($\Delta = 18.6 \text{ mJ}\cdot\text{m}^{-2}$), indicating a more damaged and energetically irregular surface. Conditioner treatment produced the lowest total surface energy (median $45.1 \text{ mJ}\cdot\text{m}^{-2}$) and the smallest heterogeneity range ($\Delta = 9.2 \text{ mJ}\cdot\text{m}^{-2}$), suggesting strong passivation (a heavy coating) but at the expense of reducing interactive sites on the hair surface. Specific surface energy (acid-base component) dropped sharply after bleaching (from 7.05 to $4.19 \text{ mJ}\cdot\text{m}^{-2}$) and reached its lowest value with conditioner ($2.19 \text{ mJ}\cdot\text{m}^{-2}$), confirming the loss of polar functionality (hair becomes less able to bond with water or treatments). *A. strigosa* complex restored specific surface energy towards virgin hair values ($4.43 \text{ mJ}\cdot\text{m}^{-2}$ at 0.5%), while maintaining dispersive energy close to untreated hair, demonstrating balanced recovery of polarity and hydrophilicity (hair regains its natural interactive properties). Overall, these results demonstrate that *A. strigosa* complex does not simply coat the hair fibre as seen with a conventional conditioner but actively improves its physical and chemical integrity.

The *A. strigosa* complex significantly reduced hair damage when applied at a concentration of just 0.2%, demonstrating a strong restructuring effect as judged by electron microscopy imaging (Figure 7, middle panel). Compared to untreated hair (Figure 7 upper panel), treatment with *A. strigosa* complex resulted in a smoother, resealed and restored cuticle layer. Damage levels were scored, and the degree of improvement was calculated, as shown in Figure 7, lower panel. Hair damage was reduced by 40% compared to the untreated sample and by 26% compared to the placebo. The ability of *A. strigosa* complex to repair the damaged hair surface is obvious in the electron microscope images.

Treatment with 0.5% *A. strigosa* complex shampoo increased gloss by more than 50% after just one treatment compared to the placebo. After the fifth washing cycle, gloss improved by 105%. The results are presented in Figure 8.

A leave-on (immersion) treatment with 0.5% *A. strigosa* complex increased tensile strength by 30%, whereas a 0.25% concentration nearly halved this effect compared to the placebo (Figure 9). Water-based immersion was used to evaluate the effect of *A. strigosa* complex without interference from other cosmetic formulation ingredients.

The overview of the effect of *A. strigosa* complex on tensile strength upon different formulations is shown in Figure 10. Treatment with 0.5% *A. strigosa* complex shampoo resulted in a 9% increase in tensile strength after just one washing cycle compared to the placebo. With additional washing and drying cycles, tensile strength remained unchanged even after the fifth cycle (data not



Decrease in hair damage

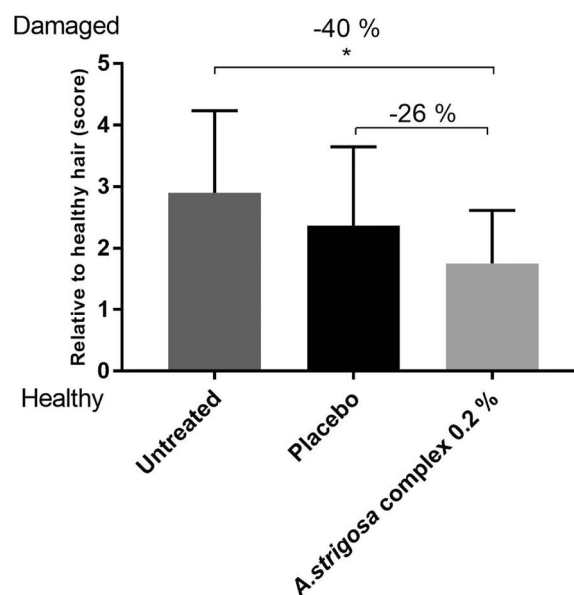


FIGURE 7 Scanning electron microscopy (SEM) pictures and quantification of hair structure. Representative example of bleached pre-damaged hair after five washing cycles without shampoo (non-treated control, upper panel) and 0.2% *Avena strigosa* complex-treated samples (middle panel). SEM pictures of at least 12 hairs per press were taken. Quantification of damage score is represented in the lowest panel.

shown). A single application of 0.5% *A. strigosa* complex in a conditioner formulation increased tensile strength by more than 13%.

Hair stressed by excessive brushing and blow-drying developed significantly fewer split ends when pre-washed with a shampoo containing 0.5% *A. strigosa* complex. Compared to the placebo shampoo, the preventative effect was 27% (Figure 11). The visual results are presented in the lower panel.

In vivo study

The shampoo containing 0.5% *A. strigosa* extract complex demonstrated superior performance after just one washing compared to the placebo for several sensory and in-use parameters (Figure 12). During washing, foam was perceived as softer and less adhesive, and rinseability improved significantly (mean difference: +0.90 points; $p=0.0017$). Foam texture and tactile sensation also showed significant enhancements (+1.10 and +1.05 points, respectively; $p<0.05$).

On wet hair, differences in detangling and combability were minor and did not reach statistical significance. After

drying, hair treated with *A. strigosa* extract complex exhibited higher ratings for visual and tactile attributes, including shine, surface smoothness and overall appearance. Suppleness and perceived vitality were also improved. Absolute ratings for dry hair quality were consistently within the 'good' to 'very good' range (mean scores approximately 7.5–8.5), with the test product outperforming the placebo in parameters such as shine, smooth surface and well-groomed appearance. No adverse effects, such as residue or build-up, were observed.

DISCUSSION

Damaged hair is characterized by the loss of amino acids and peptide fragments, which compromises the integrity of keratin structures and leads to increased brittleness. Our findings confirm this phenomenon and demonstrate that selective uptake of specific amino acids can complement these losses, restoring structural cohesion.

Evidence for this mechanism is provided by the interaction of *A. strigosa* extract with keratin, particularly KRT85. The observed decrease in melting temperature (T_m) indicates that the extract interacts with hydrogen bonds within secondary structures, suggesting penetration beyond the cuticle into deeper regions of the fibre. This molecular interaction translates into macroscopic improvements: surface energy and sorption constants approach values typical of healthy hair, indicating a more compact and less porous structure.

Electron microscopy corroborates these findings, revealing resealed cuticles and reduced porosity. Gloss measurements confirm the visual improvement, while thiol accessibility experiments demonstrate reduced exposure of reactive groups, consistent with a tighter structural network. Importantly, tensile strength increased by up to 30% in leave-on treatments, despite

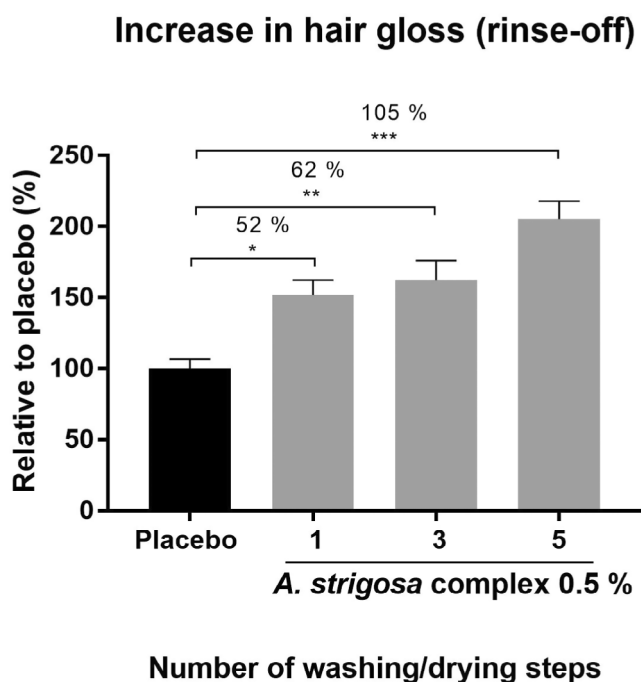


FIGURE 8 *Avena strigosa* complex improves hair gloss in a rinse-off treatment. Results of gloss measurement after different washing/drying cycles with *A. strigosa* complex shampoo.

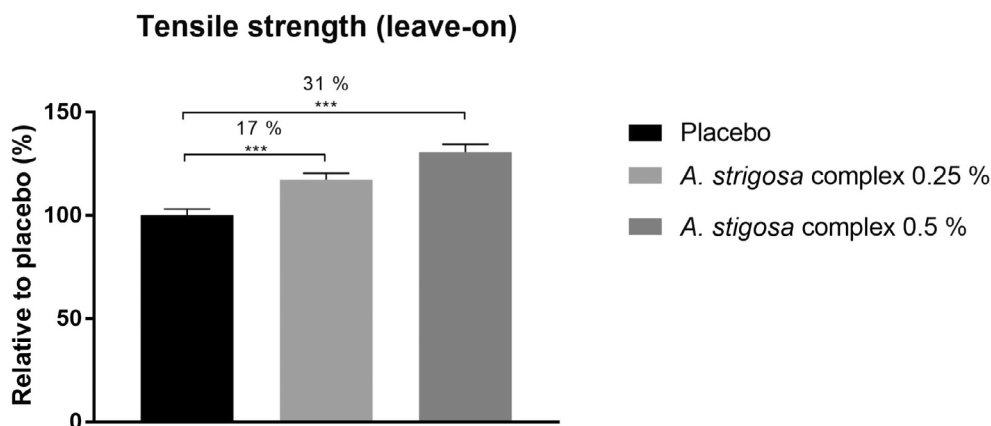


FIGURE 9 *Avena strigosa* complex dose-dependently increases hair tensile strength in leave-on treatment. Results of measurement of tensile strength after immersion with 0.25% and 0.5% *A. strigosa* complex.

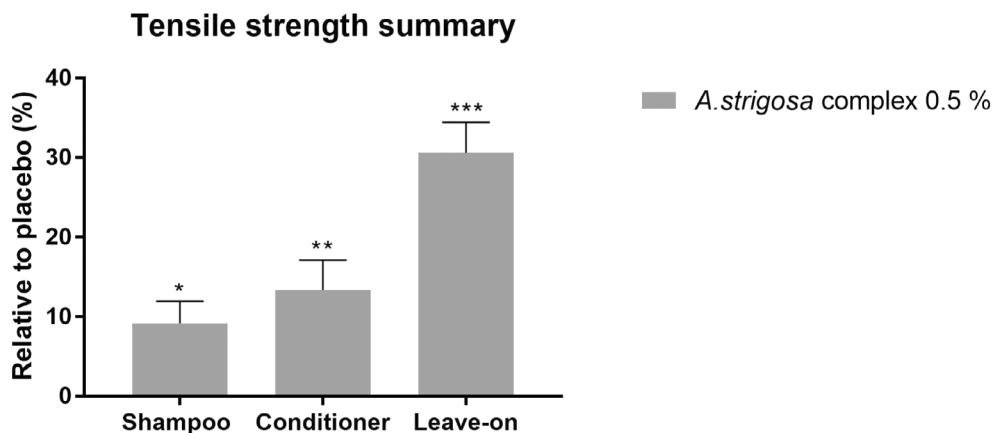


FIGURE 10 Effect of *Avena strigosa* complex on tensile strength of damaged hair following application of different cosmetic formulations. Tensile strength was measured after treatment with shampoo, conditioner, and leave-on formulations containing 0.5% *A. strigosa* complex.

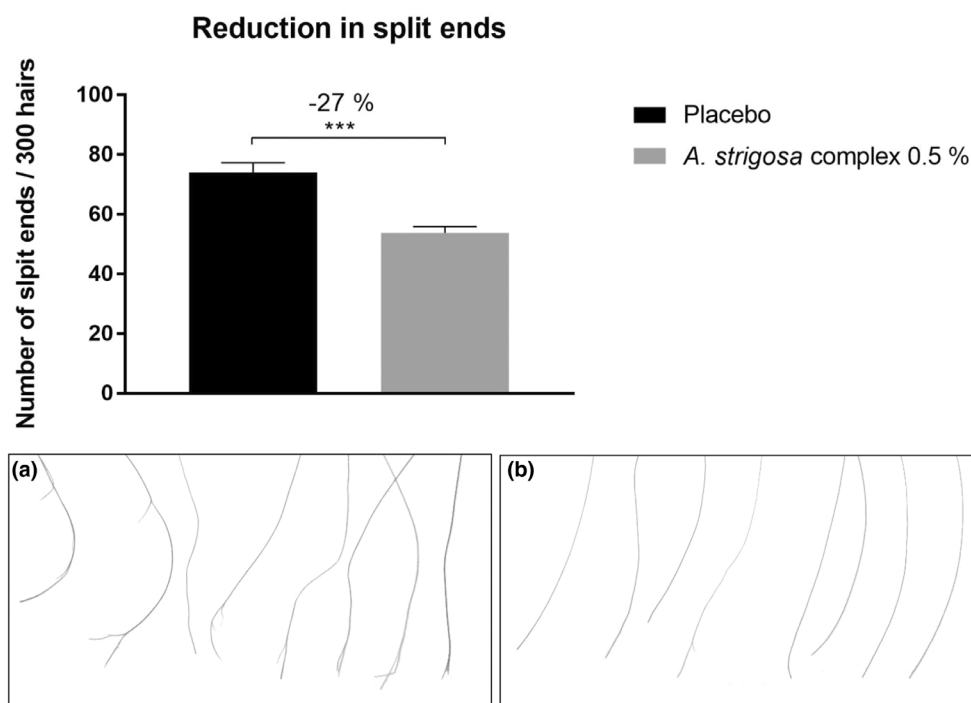


FIGURE 11 *Avena strigosa* complex reduces the formation of split ends. Stressed hair that was pre-treated with placebo showed on average 74.0 split ends per 300 hairs (24.7%) compared to 53.8 split ends per 300 hairs (17.9%) for hair that was pre-treated with 0.5% *A. strigosa* complex. Illustrative digital images are shown in the lower panel. (a) Hair selected from a strand pre-treated with placebo. (b) Hair selected from a strand pre-treated with 0.5% *A. strigosa* complex.

the apparent contradiction with T_m reduction. This discrepancy can be explained by the aqueous measurement system: within the hair fibre, integrated amino acids reinforce the hydrogen-bond network rather than destabilize it.

From a consumer perspective, these structural improvements translate into fewer split ends, enhanced tactile properties during washing and overall better hair aesthetics. Both rinse-off and leave-on formulations

proved effective, with leave-on treatments offering superior repair for severely damaged hair, while rinse-off products are suitable for maintenance. To further strengthen the consumer relevance of these findings, future work could include visual presentation of in vivo sensory attributes such as shine, smoothness and well-groomed appearance.

Collectively, these results highlight the concept of keratin intra-bonding, reinforcement of non-covalent

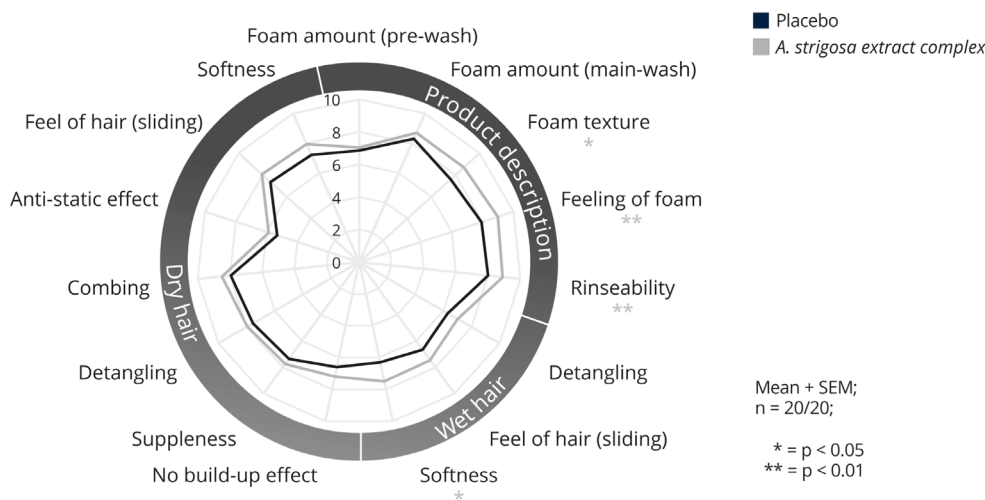


FIGURE 12 *Avena strigosa* extract complex improves the hair dressing parameters of stressed hair. Comparison of a shampoo with and without 0.5% *A. strigosa* extract complex in a split-head test.

interactions and preservation of disulphide integrity as a viable strategy for restoring hair strength and appearance using a natural, sustainable active.

CONCLUSION

This work establishes a mechanism-driven approach to repairing chemically damaged hair using a plant-derived amino acid complex from *A. strigosa*. By reinforcing keratin intra-bonding and preserving disulphide integrity, the treatment delivers measurable improvements in strength, gloss and surface compactness. These findings highlight the potential of bio-based actives to move beyond superficial coating towards structural restoration. A future step to enhance translational relevance would be the evaluation of long-term in vivo performance under real-life usage conditions. A suitable approach could include a randomized, controlled study on volunteers with chemically or thermally damaged hair over several weeks, assessing cumulative repair and wash-out resistance through instrumental measurements, expert grading and subject-reported outcomes. Such data would help confirm the durability and consumer relevance of the keratin-targeted repair observed in vitro and ex vivo.

AUTHOR CONTRIBUTIONS

The manuscript was written through contributions of all authors. All authors have given approval to the final version of the manuscript.

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CONFLICT OF INTEREST STATEMENT

None.

DATA AVAILABILITY STATEMENT

Research data are not shared.

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