An *ex vivo* comparative study of the tensile strengthening efficacy of protein-derived actives on heavily bleached hair

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Introduction

Chemical hair lightening remains a current fashion and beauty trend which shows no signs of decline. However, consumers are often distressed to discover that the quality of their hair has been significantly impaired by the harsh chemical process, with breakage proving to be a major source of concern. Whilst conventional conditioning agents effectively manage cuticular damage, the reversal of **cortical weakening** of hair poses a greater challenge. **Protein-derived actives** have been shown to confer cortical strengthening by improving axial strength and elasticity of hair fibre [1]. For example, Roddick-Lanzilotta et al. [2] have shown significant improvement **of tensile strength** of permed hair by conditioner formulations containing 1% and 3% of collagen hydrolisate combined with glycoproteins. Schulze-Zur Wiesche et al. [3] investigated a new keratin active derived from a microfibrillar "low-sulfur"- cortex keratin of wool and demonstrated that the active effectively reduces the aging effects on the human hair structure. However, the molecular features important in mediating the repairing responses remain unclear.

Aim

The aim of this study was to compare the relative efficacy **of hydrolised wheat protein (HWP), L-arginine (Arg) and hydrolised collagen (HC) in improving wet and dry tensile properties of heavily bleached hair tresses**.

This study was performed *ex vivo* using active-treated and active-untreated hair tresses as the substrate. Treatments included conditioning formulations with incorporated actives.

Materials and Methods

Formulae:

A hair conditioning cream containing **cetearyl alcohol, paraffinum liquidum, glycol distearate, steareth-21, glycerine, water and preservatives** was formulated as a vehicle for the protein-derived actives. The pH was adjusted by citric acid solution to 4 – 6 after incorporation of active ingredients. Investigational protein derivatives were: **hydrolysed wheat protein (HWP), L-arginine (Arg) and hydrolysed collagen (HC).** Each active ingredient was incorporated at five concentrations**: 1, 2.5, 5, 10 and 12.5%** (w/w as supplied), in order to establish the concentration-response profiles.

Hair substrate:

Caucasian hair tresses from the same head, approximately 13 cm in length and 4.4g in weight were used. Tresses were bleached at 12% H_2O_2 for 90 minutes and rinsed for 3 minutes under running water at ca. 40°C. Further, bleached tresses were blow-dried at 50°C for 5 minutes, with combing at an approximate rate of 60 strokes per minute. Once dried, tresses were further damaged using ceramic straighteners at temperatures of over 180°C, for 5 minutes at a rate of 6 strokes per minute.

Hair treatment:

The treatment method of tresses with conditioner was as follows (method adapted from Assmus et al [4]):

- Bleached and groomed hair tresses were washed for 5 minutes with 8% sodium lauryl ether sulphate and rinsed for 3 minutes with water (ca. 40°C).
- Tresses were treated with conditioner formulation, using 1g/g of hair, and massaged for the required period of time without combing.
- Tresses were rinsed for 3 minutes with water (ca. 40°C) and finger-squeezed dry.
- 'Wet' instrumental measurements were carried out.
- Tresses were dried with a hairdryer at 50°C for 5 minutes without combing.
- 'Dry' instrumental measurements were carried out.

Figure 1. Bleached tress (upper) and the same tress before bleaching(lower picture).

Untreated hair tresses were used as controls. Tensile tests were performed **60 minutes** after the product application. For the time-response profiles, the treatment times were **5, 10, 15, 30 and 60 minutes.**

Tensile strength measurement:

Tensile break testing was performed on the TA.XT Plus Texture Analyser and results were recorded using the Texture Exponent Software (Stable Micro Systems Ltd, UK).

No industry standards exist for tensile testing methods, therefore supplier recommended settings were utilised. The tensile testing method was carried out as follows:

- Individual hair strands of 55 mm in length were secured on mounting cards and clamped in the tensile grips (AG/T) of the Texture Analyser.
- The load was applied vertically to the long axis of the fibre at a rate of 0.5mm per second until breakpoint.
- The extension of the fibre (mm) was plotted against force (N) on the Texture Exponent Software and the force at breakpoint was recorded.

Statistical analysis:

Two-way and one-way ANOVA , as appropriate, followed by post-hoc Tukey Honest Significant Difference Test (THSD) was employed to analyse the data. Probability result of $p < 0.05$ were considered statistically significant

Results and Discussion

Tensile strength of control hair tresses:

As expected, intensely bleached tresses ware weaker than virgin hair. Two-way ANOVA revealed a highly significant difference ($p= 0.0023$) between the tensile strengths of wet and dry bleached hair. In the **wet state**, bleached hair was on average 43% weaker than virgin hair ($p<0.002$), while in the dry state it was approximately 35% weaker ($p= 0.02$). Overall, the findings provided confidence in the tensile testing method and established the upper and lower test limits for the virgin and bleached tensile readings, respectively.

Concentration response of 60-min treated hair tresses:

The results for the wet fibre tensile break strengths are presented in Figure 2a, whilst the dry results are presented in Figure 2b.

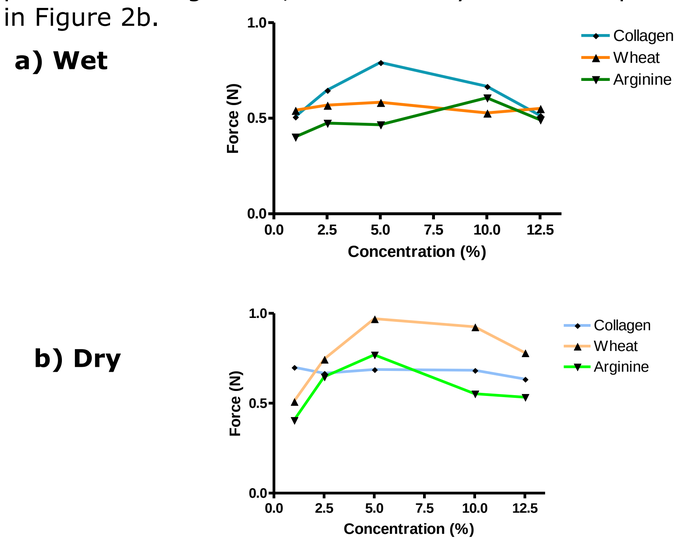

Figure 2. Tensile strengths of active-treated bleached hair in the wet state (a) and dry state (b) as a function of concentration (n=6, 60 minutes treatment)

The greatest wet hair strengthening properties were obtained at 5% for HPW and HC, and at 10%for Arg. In the dry state, **HWP** solution evoked a maximal response at 5% active formulation, mirroring the findings from the wet analysis, hence **5%** was employed for subsequent testing.

Arginine evoked the greatest mean response at 5% concentration for dry fibres, challenging the findings from the wet analysis. The results from 2-way ANOVA and THSD revealed that the concentration of 5% Arg evoked a larger improvement in tensile break strength relative to a 10% concentration, hence **5%** was chosen.

HC evoked the greatest mean response for dry fibres at 1% concentration, again in conflict with the findings from the wet results. THSD revealed that a **5%** HC formulation provided the greatest increase in bleached fibre tensile strength for both wet and dry hair overall, and this concentration was used in further study.

The impact of treatment time:

The tested times included: 5, 10, 15, 30 and 60 minutes.

For wet hair (Figure 3a), HWP and HC showed an increase in tensile break strengthening with increased exposure time up to 60 minutes. Conversely, Arg was observed to peak at an optimal exposure time of 15 minutes.

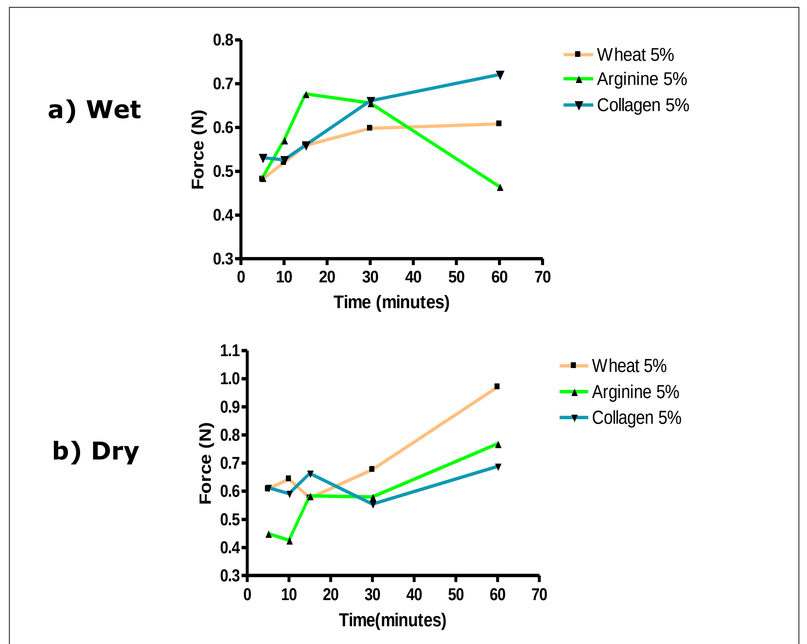

Figure 3. Tensile strengths of active-treated bleached hair in the wet state (a) and dry state (b) as a function of time (n=6)

The overall strengthening property of HWP and HC in the dry state showed again an increase with increasing time, while Arg exhibited similar behaviour, contrary to that displayed for wet hair.

Arg was statistically most effective at treating wet and dry tresses after 60 minutes, in comparison with the other actives. However, this was not the trend observed in the instance of wet fibre strengthening properties. This point clearly requires further work.

An increase in response with increasing exposure time suggests that penetration into the fibre is the rate-limiting step of the active/keratin association. In order to reach the cortex, the active molecules must firstly show affinity for the cuticle and then penetrate beyond the cuticle via either a transcellular or intercellular route.

Transcellular diffusion requires selective uptake of the active into the cuticle and cortical cell membranes and consequent excretion until target cortical sites are reached. Diffusion via the intercellular route offers a less energetically demanding solution for the gain of cortical access. Nonetheless, the route to the cortex is labyrinthine and thus it is to be expected that an increase in exposure time will result in a higher chance of active molecules reaching their target cortical sites.

The graph would be expected to plateau once the active molecules had saturated the available cortical binding sites, which appears to be in the time longer than 60 minutes. This theory is supported by a certain levelling of the responses for wheat and collagen in the wet states, however investigations with longer exposure times are required to confirm this proposition.

Conclusion

Overall, the most efficacious of the protein-derived actives, taking into account both wet and dry tensile tests, was found to be **hydrolysed wheat protein** ($p < 0.0001$), closely followed by hydrolysed collagen ($p<0.01$). L-arginine did not significantly improve overall tensile strength versus bleached control tresses. It is suggested that the mechanism of action in conferring tensile strengthening properties to damaged hair fibres involves the substitution of hydrogen and electrostatic bonds in the cortex.

References

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Acknowledgments

The authors wish to acknowledge the excellent technical assistance of Mrs Carmel Lally and Mrs Senait Tewolde. This work is presented at the IFSCC Paris 2014 Congress with financial support from the Society of Cosmetic Scientists (United Kingdom).

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