SCIENTIFIC PAPER

# An *ex vivo* Comparison of the Tensile-Strengthening Properties of Protein Derivatives on Damaged Hair

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## ABSTRACT

*This study aims to compare the effects of three protein-derived conditioning actives (keratin, wheat and collagen hydrolyzates) on the tensile strength of three types of damaged hair: bleached, permed and thermally styled. The investigated actives were hydrolyzed keratin (600 Da), hydrolyzed wheat protein (and) wheat starch (1,500 Da) and hydrolyzed collagen (9,000 Da). Each active was incorporated into an emulsion base containing cetrimonium chloride at 1% w/w, thus providing three active conditioning treatments. Virgin Caucasian brown hair tresses were damaged following standardized bleaching, perming and repetitive thermal straightening protocols. The three active treatments and a control were applied to each type of damaged hair tress under controlled conditions. The tensile strength of both wet and dry hair fibers was measured before and after each treatment using the TA.XT Plus Texture Analyzer (Stable Micro Systems, Godalming, UK).*

*The results have shown that all three types of damage treatments reduced the tensile strength of hair in both wet and dry states, with wet damaged tresses showing significantly lower tensile strength than their dry counterparts. Hydrolyzed keratin was shown to improve the wet tensile strengths of all three types of damaged hair, thus emerging as the most efficacious treatment for wet hair, followed by hydrolyzed collagen. Hydrolyzed wheat protein and wheat starch were shown to be most effective in improving the tensile strength of dry hair, especially if bleached or thermally treated.*

## INTRODUCTION

The remarkable mechanical resilience of hair fiber is due to its complex protein structure enforced by multiple types of bonds: covalent bonds (disulfide and isopeptide bonds), ionic bonds, hydrophobic forces, and hydrogen bonds [1]. Tensile strength, as one manifestation of the overall mechanical properties of hair, has been studied extensively and is best described by the strain/stress curve of a single hair fiber [2]. The fiber elongation (strain) as a result of continuous extension force (stress) exhibits distinct regions of elastic and thixotropic behavior, which are significantly influenced by the humidity conditions [2, 3]. While the precise impact of all bond types on the tensile strength of hair is not yet fully understood, the role of disulfide bonds in particular has been researched extensively in relation to bleaching and permanent waving of hair [3]. Disulfide bridges (bonds) across the amorphous proteins in the cortex create a matrix within which the crystalline microfibrils are embedded and stabilized. Due to permanent conversion of cystine to cysteic acid in the bleaching process, the degree of cross-linking in the matrix is expected to be reduced. *Robbins* and *Kelly* [4] documented decreases of cystine in samples of hair bleached under at-home and laboratory conditions by up to 25% and 45% respectively, with a corresponding increase in cysteic acid. Permanent waving, on the other hand, aims to reduce the disulfide bonds in the cortex in order to enable a realignment of the crystalline structures, after which the application of an oxidizing agent aids the formation of new disulfide bonds. About 20% of cystine is reduced in the first stage of the process but is then largely recovered in the oxidation stage. The levels of free cysteic acid in permanently waved hair following both laboratory and at-home treatments were reported by *Robbins* and *Kelly* to be substantially below those found in bleached hair [4]. It is, therefore, expected that the different chemical treatments will impact differently on the mechanical properties of hair.

Following the increased popularity of high temperature (> 185 °C) styling appliances the impact of high temperature on the structure and mechanical properties of hair became a subject of investigation. *Milzarek et all.* [5] linked the exposure of hair at increasing temperatures (90-180 °C) to the progressive loss of water and the gradual replacement of hydrogen bonds with stronger ionic bonds. This process affects both crystalline and amorphous regions in the cortex and initially leads to a partial degradation of structures. However, at T > 155 °C an irreversible recrystallization occurs which forms new protein phases largely inaccessible to water and with different mechanical properties [6].

Tensile strength measurements based on stretching a fiber of a known length at a fixed rate were first found to be sensitive to changes in the hair condition caused by bleaching and cold waving in the 1960s [7]. Such measurements describing the hair elasticity and strength remain a helpful method for analysis and quantification of the impact of grooming and environmental factors on the structural integrity of hair. Specifically, the decrease in force required to break a single fiber would infer structural cortex damage, thus reflecting the changes to covalent (disulfide) and strong ionic bonding within and between the crystalline and the amorphous regions of the cortex [3, 8, 9]. While such measurements cannot be directly related to the capacity of hair to withstand the forces involved in the grooming process, they could contribute to the understanding of hair damage and repair processes.

Protein-derived conditioning actives share similarities with the amino acid structure of the hair keratin and have been found to enhance the tensile strength of damaged hair [9-12]. The natural affinity of these ingredients to the hair is due to their capacity to bond ionically and via hydrogen bonding with the amino acid residues of the hair proteins, thus maintaining the water content in the cortex and the stability of internal structures. Hence, protein hydrolyzates and peptide mixes are theoretically the preferred choice of hair treatments for chemically and thermally damaged hair. The interactions of protein actives and the hair substrate are dependent on the isoelectric point and pH [13]. Hence, as the nature of the chemical composition and isoelectric point of damaged hair varies, it is possible that the differences in the amino acid profiles and molecular weight of the treatment actives could result in differences in their repair efficacies. Specifically, this study aims to compare the effects of keratin, wheat and collagen hydrolyzates on the tensile strength of three types of damaged hair: bleached, permed and thermally treated

## EXPERIMENTAL

### Materials

Protein hydrolyzates with different amino acid profiles, reflecting their different origins (plant or animal) and with different molecular weights, were selected for this investigation. Their INCI designations, molecular compositions, molecular weights and optimal pH ranges are listed in *Table I*.

To make conditioner samples, each active investigated was incorporated into a stable cationic emulsion base ***(Table II)*** in the following concentration levels:

- hydrolyzed keratin (referred in further text as "keratin") - 0.5% w/w,
- hydrolyzed wheat protein (and) wheat starch (referred to as "wheat") - 2% w/w and
- hydrolyzed collagen (referred to as "collagen") - 2% w/w.

These concentration levels for the test actives were identified in a preliminary concentration response study based on the suppliers' recommendations and employing the same testing method used in the main study. The pH of all test formulations was adjusted to 4.0 (+/-0.2).

Caucasian virgin hair tresses weighing 1.5 g (+/-0.1g) and 150 mm in length were used as the treatment substrates.

*Table I* Protein-Derived Conditioning Actives.

| Source/ Derived from* | INCI name (% active as supplied) | Average molecular weight * (Da) | pH * |
|---|---|---|---|
| **Animal/ Wool** | **Hydrolyzed keratin (20%) **** highly fibrous proteins with content and structure close to human keratin (α-helix) | 600 | 4.0-5.0 |
| **Plant/Wheat** | **Hydrolyzed wheat protein *** (and) wheat starch (85%)** mono and polymeric prolamines + mono and oligosaccharides | 1,500 | 4.0-5.0 (in 10% water) |
| **Animal (bovine hide) /collagen** | **Hydrolyzed collagen (95%) ***** tropocollagen formed by triple helix structures | 9,000 | 5.5-6.5 (in 10% water) |

* Information as provided by respective suppliers
** Supplier: Provital Group, Barcelona, Spain
*** Supplier: Croda Personal Care, Snaith, UK

*Table II* Composition of the Cationic Base Emulsion (Control Conditioner).

| INCI | Function | Supplier | % w/w |
|---|---|---|---|
| Aqua | Solvent | n/a | to 100 |
| Glycerin | Humectant | Sigma Aldrich, Gillingham, UK | 3.00 |
| Cetrimonium chloride (30%) & aqua | Monoalkyl cationic conditioner and emulsifier | Azelis UK Life Sciences Hertford, UK | 1.00 |
| Cetearyl alcohol | Nonionic emulsifier | Surfachem, Leeds, UK | 5.00 |
| Ceteareth-20 | Nonionic emulsifier | Azelis UK Life Science, Hertford, UK | 2.00 |
| Propylene glycol (and) diazolidinyl urea (and) methylparaben (and) propylparaben | Preservative (carried in propylene glycol solvent) | Azelis UK Life Science, Hertford, UK | 0.50 |
| Citric acid (20%) | pH regulator | Sigma Aldrich, Gillingham, UK | q.s. |

Methods

*Hair damage treatments*
Bleaching: Four hair tresses were treated according to the manufacturer's instructions for 60 minutes with a commercial professional hair bleaching product containing 12% w/w $H_2O_2$.

Permanent waving: Four hair tresses were treated with a commercial professional hair perming kit comprising a perm lotion and a neutralizer for "normal and resistant" hair. The active levels of ammonium thioglycolate and hydrogen peroxide were not indicated by the supplier.

Repetitive thermal straightening: Four hair tresses were treated following a modified published protocol for heat treatment [6] comprising four consecutive cycles of washing, blow drying and intermittent applications of a flat straightening ceramic iron (210 °C) for a total of 3 minutes [6].

*Tensile strength testing*
Following the final rinse six wet single hair fibers were selected randomly from each damaged/treated wet tress and removed for immediate wet tensile strength testing. The hair tress was then blow-dried at 50°C for 5 minutes, and six single fibers were removed for dry tensile strength testing. A Texture Analyzer TA.XTPlus equipped with Texture Exponent software (Stable Microsystems, Godalming, UK) was used to perform all tensile tests. A single hair fiber (length=55 mm) was stretched at a constant speed of 15 mm/s and the force that caused the hair to break was recorded. A typical single hair fiber force/extension plot is presented in ***Figure 1***. The plot is not dissimilar to the stress-strain curve commonly used to describe the mechanical properties of hair fibers, with the region A-B representing elastic behavior of the hair, B-C representing the yield region representing thixotropic behavior and C-D the nonelastic deformation leading to the break of the fiber [8].

The test conditions and analysis for normality satisfied the conditions for parametric statistical analysis. The breaking force results were therefore analyzed using a

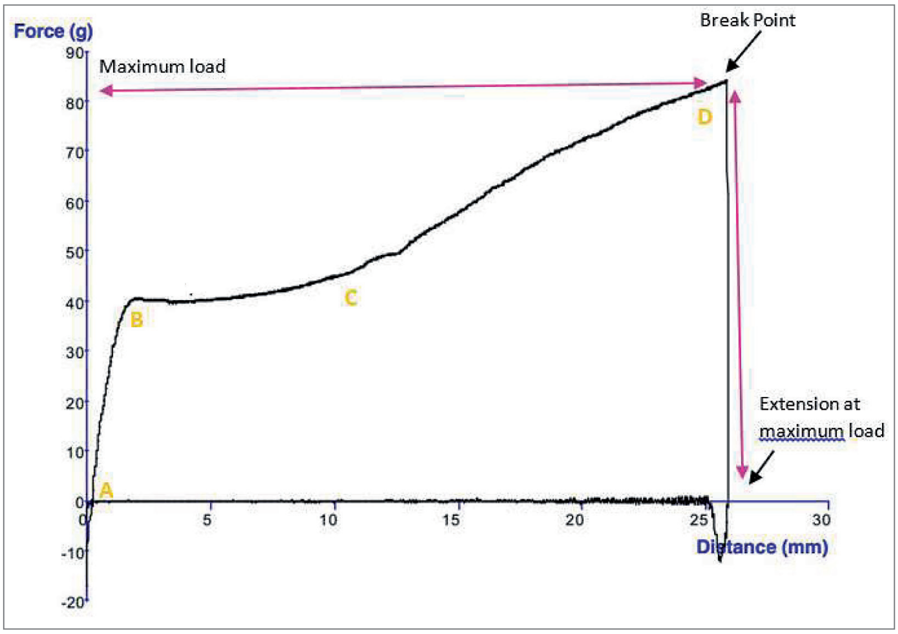


*Figure 1* Typical tensile strength plot of a single hair fiber obtained by a Texture Analyzer TA.XTPlus equipped with Texture Exponent software.

two-away analysis of variance (ANOVA) (factor 1= wet/dry state, factor= type of treatment), followed by a Tukey honestly significant difference (THSD) test. The R programming language (R Foundation for Statistical Computing, Vienna, Austria) was used for all statistical data analysis. Results with a 95% confidence level, (alpha = 0.05) have been reported.

*Conditioning treatments of damaged hair*
A quantity of 2 ml of sodium laureth sulfate solution (27% w/w) was applied to a damaged hair tress, massaged in for 30 seconds and rinsed out under running water (35 °C) for 1 minute. A conditioning treatment with the test formulation (0.7 g per 1 g of hair) was then applied, massaged in for 30 seconds and left on the hair for 5 minutes. Finally, the tress was rinsed for 1 minute under running water (35 °C). Both wet and dry tensile strength tests were performed using the above test protocol. Hair tresses representing each hair damage type were subjected to four repair treatments – a control (cationic emulsion base) and three active treatments (containing different protein hydrolyzates). In total, twelve variations of hair damage and respective repair treatments were measured for each state (wet and dry).

*Conditioning treatment of bleached hair at elevated temperature*
The above protocol was applied with the following modification: The treated hair tress was placed in a towel steamer at 104 °C for 5 minutes.

## RESULTS AND DISCUSSION

*Tensile strength of damaged hair*
The breaking force values for virgin, bleached, permed and thermally strengthened hair in both wet and dry states are displayed in ***Figure 2***. Statistical analysis of all damaged hair types (wet and dry) and virgin hair confirmed a statistically significant difference in the sample ($p \leq 0.05$). The THSD paired comparison test indicated that differences existed between each respective damage type and virgin hair in both the wet and dry states and between the wet and dry state of each hair type.

The reductions in tensile strength of wet bleached and permed hair in comparison with their respective dry states have been attributed to increased exposure of the cortex to water. It is assumed that some cortex areas previously inaccessible to water are chemically altered by the bleaching. The new structures result in disruptions of hydrogen bonds, thus lowering the wet tensile properties of fiber to

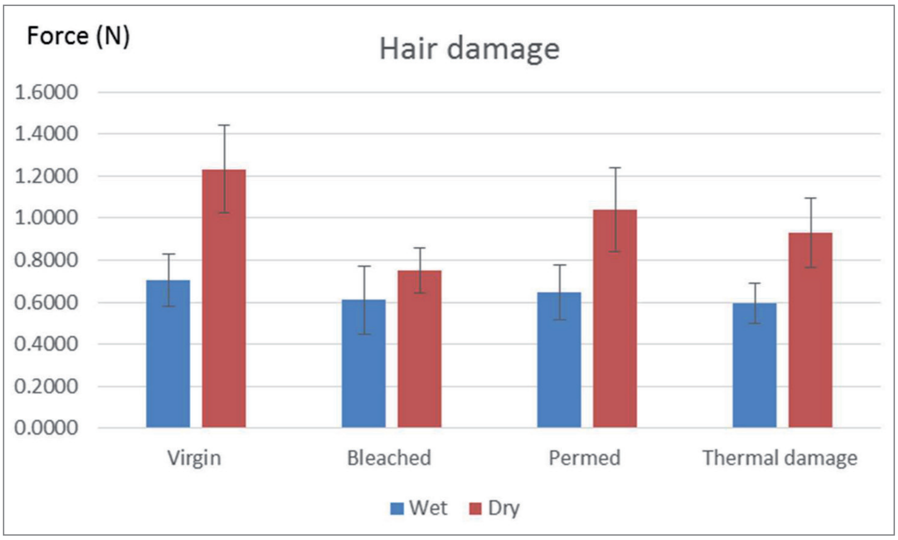


*Figure 2* Comparison of the mean breaking force of virgin, bleached, permed straightened hair in the wet and dry state. The bars display mean values and confidence intervals at the 95% confidence level.

a greater extent than in the dry state [3]. The difference obtained in dry breaking strength between bleached and permed hair (statistically significant) can be explained by the high and irreversible loss of cystine in the bleached hair, possibly accompanied by additional chemical hydrolysis of proteins, leading to the formation of less crosslinked protein structures. Such extent of chemical changes in the permed hair is unlikely due to the controlled reformation of cystine, hence its higher dry tensile strength. For thermally straightened hair, the results showed a similar large decrease in breaking force for both wet and dry states. These results are in line with the predicted increased crystallinity and decreased water-binding capacity of the thermally damaged fiber, hence giving the smaller difference in tensile strength reduction between wet and dry states.

In summary, the tensile strength tests on the laboratory-damaged hair tresses indicted that the breaking force of hair fiber reflects the nature and degree of chemical and structural alterations caused by different types of processing.

*Tensile strength of damaged hair after conditioning treatments*

Statistical analysis of the tensile strength of hair after conditioning treatments was carried out to determine the most efficacious active for each hair damage type. The test results for all protein active treatments were compared statistically to untreated damaged hair and are presented in *Figure 3 a-f*.

Results for bleached tresses

*(a) Comparison with untreated bleached hair*

The results presented in *Figure 3 a, b* show that treatment of hair tresses with the control conditioner did not significantly improve the tensile strength, while most active treatments did (in both the wet and dry states). The keratin treatment produced the highest increase in break point force in both the wet and dry states ($p \leq 0.001$ and $p \leq 0.0001$, respectively), while the wheat and collagen treatments had the most substantive impact on the dry state of breached hair ($p \leq 0.001$ and $p \leq 0.0001$, respectively).

*(b) Comparison with cationic base emulsion*

The results of the wet and dry tests after active treatments were also compared with those for the cationic base emulsion (control) treatment. No statistically significant differences were found between the control and wheat or the control and collagen, while a significant difference was found between the wet control and wet keratin ($p \leq 0.05$). In summary, the results for bleached hair indicate that keratin is the most efficacious active (in both the wet and dry states) at a concertation level below that of the other two actives, a result which implies strong affinity to the protein chain residues of the hair fiber.

Results for permed tresses

*(a) Comparison with untreated permed hair*

The results presented in *Figure 3 c, d* show that the keratin treatment produced the most notable tensile strength increase in the wet state followed by collagen ($p \leq 0.0005$ and $p \leq 0.001$, respectively) but had no impact on dry permed hair. The wheat treatment resulted in significant tensile strength improvement in both the dry and wet state ($p \leq 0.05$).The control treatment showed no statistically significant difference compared with the untreated permed hair.

*(b) Comparison with cationic base emulsion*

Statistical differences from the control were found for all treatments: keratin ($p \leq 0.001$), wheat ($p \leq 0.000$) and collagen ($p \leq 0.05$). The results suggest that the wet hair tensile strength of permed hair could be improved by all investigated protein actives. However, the most effective overall treatment for permed hair was found to be wheat protein, improving the tensile strength of hair in both the wet and dry state.

Results for thermally damaged tresses

*(a) Comparison with untreated thermally damaged hair*

All treatments *(Figure 3 e, f)* produced statistically significant improvements in breaking force in the wet state, with that of the keratin treatment being the most notable ($p \leq 0.0005$). In the dry state, wheat produced the highest results ($p \leq 0.005$) followed by collagen ($p \leq 0.05$).

*(b) Comparison with cationic base emulsion*

Significant differences were found for all active treatments ($p \leq 0.05$), but only in the wet state.

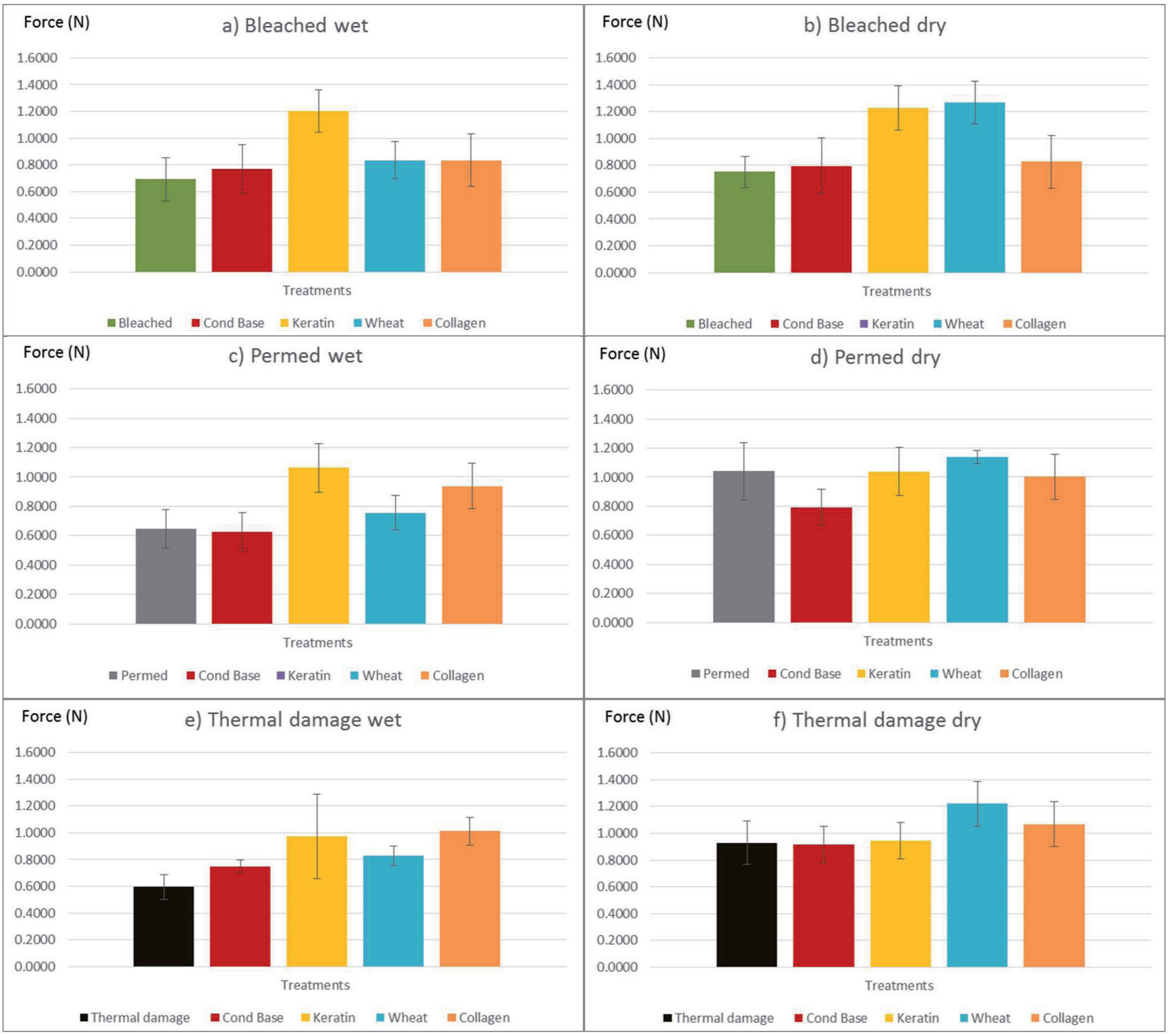


*Figure 3* Comparison of mean breaking force (N) measurements for wet and (a,b), wet and dry permed hair (c,d) and wet and dry thermally treated hair (e, f). The bars display mean values and confidence intervals at the 95 % confidence level.

### Results for bleached tresses exposed to heat during treatment

Bleached tresses were also treated at elevated temperature with all actives at the optimal concentrations used in this study (keratin - 0.5%, wheat - 2% and collagen - 2%) and the results compared with those obtained at room temperature. All results are presented in ***Figure 4 a, b***.

The wheat and collagen treatments showed statistically significant increases in breaking force in the wet state with exposure to elevated temperature ($p \leq 0.05$) ***(Figure 4a)***.

In the dry state ***(Figure 4b)***, the tresses treated with keratin and collagen at elevated temperature showed a statistically higher tensile strength than those treated correspondingly at room temperature ($p \leq 0.05$).

In summary, the temperature response data showed that the effect of the collagen treatment of bleached hair was enhanced by elevated temperatures, while that of keratin treatment was not.

Further investigations with the other protein actives are recommended.

## CONCLUSION

The aim of this study was to evaluate the efficacy of hydrolyzed proteins in improving the tensile strength of damaged hair fibers expressed as the breaking force in a tensile test.

Hydrolyzed keratin, the active with the smallest molecular weight and chemical composition most similar to that of human keratin, improved most notably the wet tensile strengths of bleached, permed and heat treated hair. Taking into consideration the lower active concentration level of hydro-

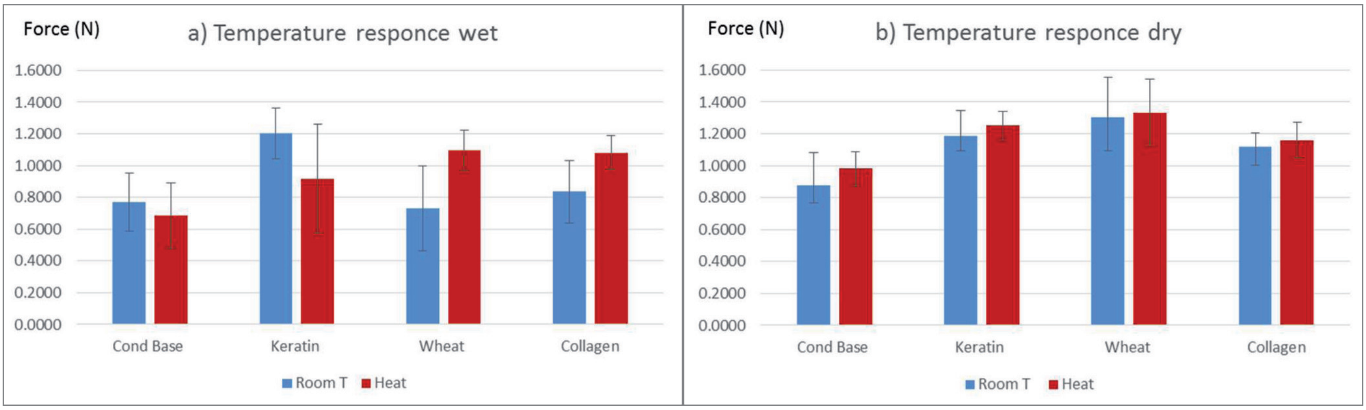


*Figure 4* Comparisons of the mean breaking force (N) measurements for wet and dry bleached hair treated with 0.5% keratin at room temperature and at 104 °C. The bars display mean values and confidence intervals at the 95% confidence level.

lyzed keratin in the treatment (0.5%) compared with that of the other two actives (2%), it emerged as the most efficacious treatment for wet hair and for bleached hair in particular. The results suggest that keratin's efficacy is aided by its molecular weight and structure as well as by the nature of the hair damage. Furthermore, the keratin treatment response to elevated temperature indicates increased mobility and weakening of bonding to the hair under those conditions.

The hydrolyzed wheat protein and wheat starch treatment containing a mixture of monomeric and polymeric peptides and oligosaccharides was most effective in improving the tensile strength of dry hair. This was especially noticeable in permed and heat treated hair, which exhibited a smaller tensile strength reduction than dry bleached hair. One explanation for this result is the presence of wheat starch in the fiber, which stiffens with drying, thus increasing the breaking force of the whole hair fiber.

Hydrolyzed collagen, the active with the highest molecular weight and a structural organization resembling the hair crystalline phases, showed the most consistent tensile strengthening effect on thermally treated hair. This effect could be due to the presence of a stronger hydrophobic attraction between the collagen and the regions of the hair cortex affected by the thermal treatment.

In summary, this study demonstrates that the breaking force is a reliable indicator of hair cortex damage caused by chemical and thermal treatments and that such damage can be mitigated by the use of optimized combinations of protein hydrolysates and treatment conditions, such as temperature.

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