Tensile Strength After Repeated Washes

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Tradename: AC Curezyme

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Lot #: N250131A

Sponsor: *Active Concepts, LLC; 107 Technology Drive Lincolnton, NC 28092*
Study Director: *Daniel Shill*
Principal Investigator: *Hannah Stade*

Test Performed:
Tensile Strength After Repeated Washes Assay

Introduction

Hair plays a crucial role in personal appearance, social identity, and overall well-being. The mechanical integrity of hair is a key determinant of perceived hair health, influencing resilience to breakage, manageability, and styling performance. Among the many external stressors that influence hair fiber structure and function, repeated washing is an underestimated contributor to cumulative hair damage. Despite the benefits of shampoo and conditioner on physical appearance and scalp hygiene, repeated washing without strengthening and protective ingredients exert a deleterious effect on the hair shaft.

Specifically, frequent wetting and drying cycles weaken the protective lipid and protein layers of cuticles as the hair shaft repeatedly expands and contracts. These fluctuations in hair shaft size led to increased porosity, moisture imbalance, and surface roughness which deteriorates the structural and functional integrity of hair. The resultant diminished tensile strength and increased susceptibility to mechanical stress and breakage of hair fibers can be exacerbated by environmental insult, surfactant exposure, and physical manipulation (e.g., towel drying, combing while wet, heat styling). This is particularly evident in hair types with pre-existing structural vulnerabilities, such as chemically treated, curly, or textured hair. Therefore, cosmetic ingredients designed to reinforce and promote healthier hair, especially in shampoo and conditioner formulations, are critical.

Accordingly, an *ex vivo* Tensile Strength After Repeated Washes Assay was conducted to assess the ability of **AC Curezyme** to protect and strengthen hair after regular washing.

Assay Principle

Virgin human hair tresses were repeatedly washed with shampoo and conditioner to understand the protective and strengthening capability of an added test article. After treatment, individual fibers were assessed to determine hair strength and resistance to deformation. Tensile properties are assessed by applying force to individual fibers until each strand breaks and force to break, tenacity, percent elongation, and elasticity are calculated. Force to break and tenacity measure hair fiber strength with higher values indicating a stronger and healthier fiber. Force to break is the maximum force required to break a fiber and tenacity reveals the breaking strength with respect to fiber linear density. Percent elongation and elasticity indicate ductility and resistance to deformation. Percent elongation provides fiber deformation before breaking and is inversely related to elasticity which expresses the ratio of change in stress to change in strain as a fraction of the original hair fiber length. More fiber elongation indicates more damage, whereas greater breaking stress indicates more elasticity.

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Materials

A. **Hair Samples:** Human Virgin Asian, Curly, Blonde, and Brunette Hair Tresses
B. **Products:** Base Shampoo and Conditioner (Table 1)
C. **Equipment:** Instron (Method ASTM D3822)
D. **Software:** Excel Analysis ToolPak (Microsoft)

Table 1. Base Shampoo and Base Conditioner INCIs and Compositional Breakdowns.

Base Shampoo Formulation	%
Water	41.0
Guar Hydroxypropyltrimonium Chloride	1.0
Sodium Methyl 2-Sulfolaurate (and) Disodium 2-Sulfolaurate	35.0
Cocamidopropyl Betaine	15.0
Lactobacillus Ferment & Lactobacillus & Cocos Nucifera (Coconut) Fruit Extract	4.0
Polysorbate 20	2.0
Fragrance	2.0

Base Conditioner Formulation	%
Water	76.0
Polyquaternium-10	1.0
Glycerin	3.0
Water & Centrimonium Chloride	2.0
Behentrimonium Methosulfate & Cetearyl Alcohol & Butylene Glycol	8.0
Hydrogenated Ethylhexyl Olivate (and) Hydrogenated Olive Oil Unsaponifiables	5.0
Lactobacillus Ferment	4.0
Fragrance	1.0

Methods

Twelve virgin hair tresses were collected and classified using the Andre Walker Hair Typing System (Image 1). Prior to any treatment, one hair tress from each hair type was assigned to the conditions described in Table 2. For each hair type, one tress received Base Shampoo and Conditioner, and a second received 2.0% **AC Curezyme** in Base Shampoo and Conditioner. All treatments were thoroughly massaged into the hair for 30 seconds before rinsing. After conditioners were rinsed out, the tresses were allowed to fully air dry. Tresses were washed 14 times with their designated treatments. An untreated tress was left unaltered as the Untreated Control for each hair type.

Image 1. Andre Walker Hair Typing System

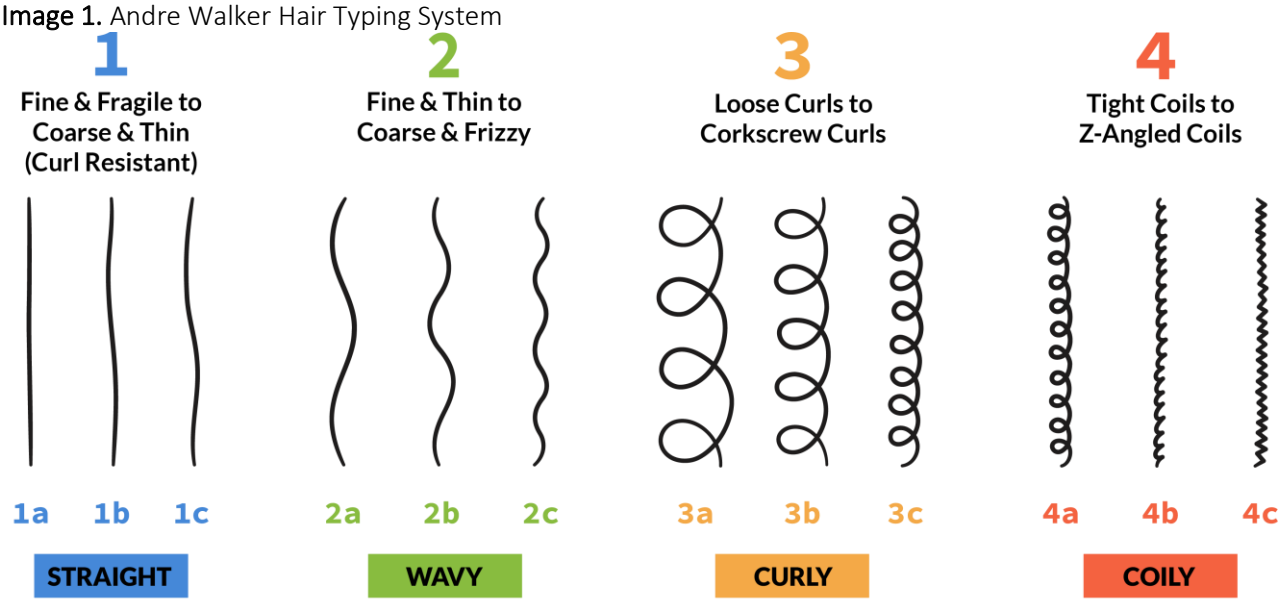

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Table 2. Descriptions of the Conditions for each Hair Type.

Condition	Treatment Description	Hair Type
Untreated Control	No Washes	1C-Straight-Black 3B-Curly-Black 1B-Straight-Blonde 2B-Wavy-Brunette
Base	14 Washes with Base Shampoo and Conditioner	
2.0% **AC Curezyme**	14 Washes with 2.0% **AC Curezyme** in Base Shampoo and Conditioner	

The Instron method ASTM D3822 was used to evaluate the effect of **AC Curezyme** on the integral structure and properties of the hair after repeated washes. Single fiber testing was performed to assess the strength of the hair in terms of force to break, tenacity, percent elongation, and Modulus of Elasticity. 25 hair strands from each tress were tested and average values were recorded. One-way ANOVAs were performed between groups with statistical significance accepted at $p \leq 0.05$.

Results

The data obtained met criteria for a valid assay as the controls performed as anticipated. For all hair types, hair tresses washed 14 times with the Base Shampoo and Conditioner demonstrated a reduction in all tensile properties tested compared to the Untreated Controls. Conversely, all hair types washed 14 times with 2.0% **AC Curezyme** demonstrated improvements in all tensile properties compared to the Untreated Controls and hair repeatedly washed with the Base Shampoo and Conditioner.

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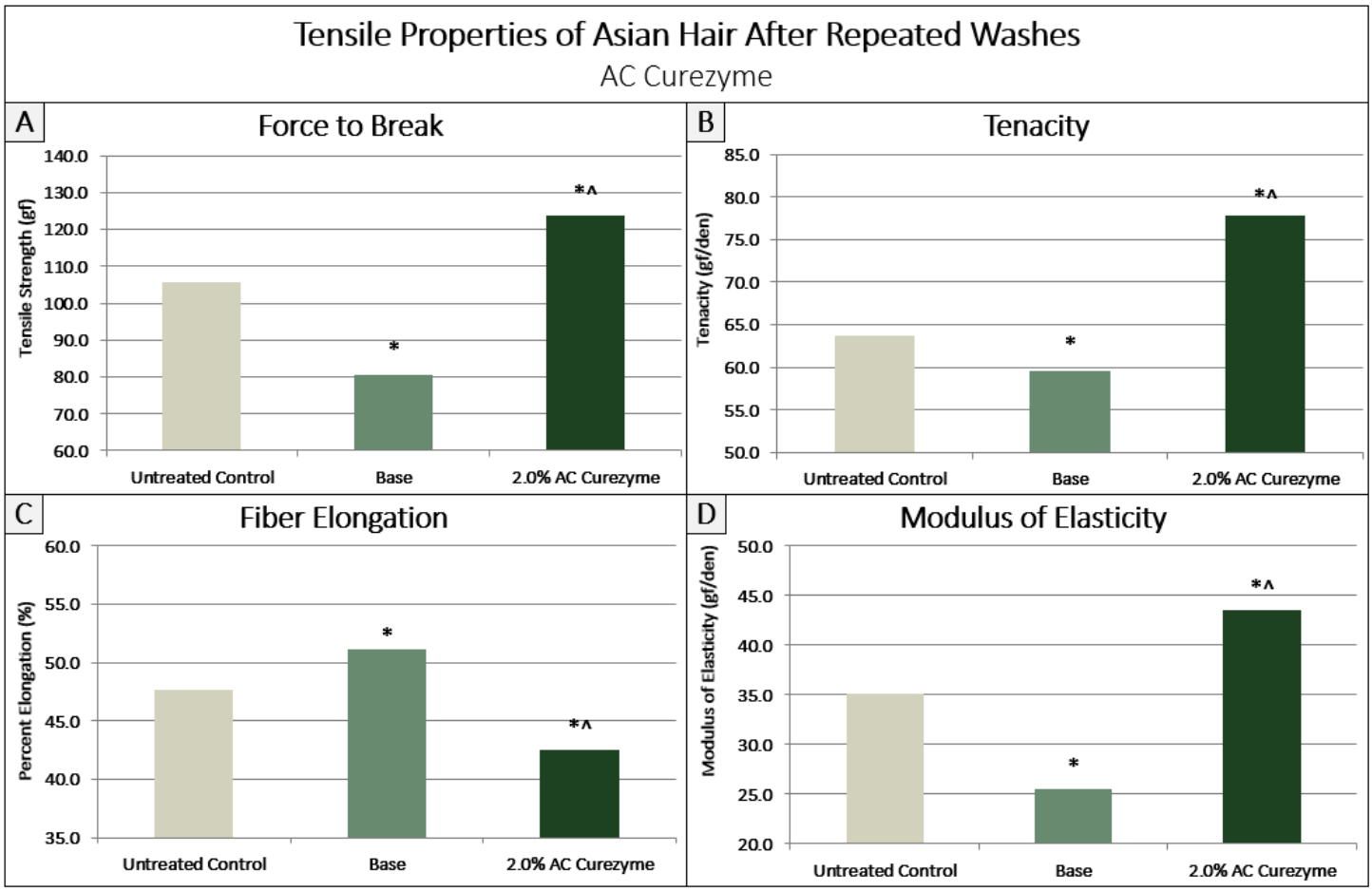

Figure 1. Average Tensile Properties of 1C-Straight-Black Tresses After 14 Washes. * indicates significance ($p \leq 0.05$) compared to Untreated Control. ^ indicates significance ($p \leq 0.05$) compared to Base (Base Shampoo and Conditioner). a) Force to Break gives the maximum force required to break each fiber where an increase indicates fiber protection, and a decrease indicates less protection. b) Tenacity gives the break strength of each fiber where an increase indicates fiber protection, and a decrease indicates less protection. c) Fiber Elongation illustrates fiber deformation before breaking where lower values indicate stronger hair fibers and higher values indicate more damaged fibers. d) Modulus of Elasticity expresses the ratio of change in stress to change in strain as a fraction of the original hair fiber where an increase indicates stronger hair fibers, and a decrease indicates more damaged fibers.

Table 3. P-values from one-way ANOVA between Conditions for 1C-Straight-Black Hair Tresses. * indicates significance ($p \leq 0.05$) compared to Untreated Control. ^ indicates significance ($p \leq 0.05$) compared to Base (Base Shampoo and Conditioner).

		Base	2.0% AC Curezyme
Force to Break	Untreated Control	< 0.05*	< 0.05*
	Base	-----	< 0.05^
Tenacity	Untreated Control	< 0.05*	< 0.05*
	Base	-----	< 0.05^
Fiber Elongation	Untreated Control	< 0.05*	< 0.05*
	Base	-----	< 0.05^
Modulus of Elasticity	Untreated Control	< 0.05*	< 0.05*
	Base	-----	< 0.05^

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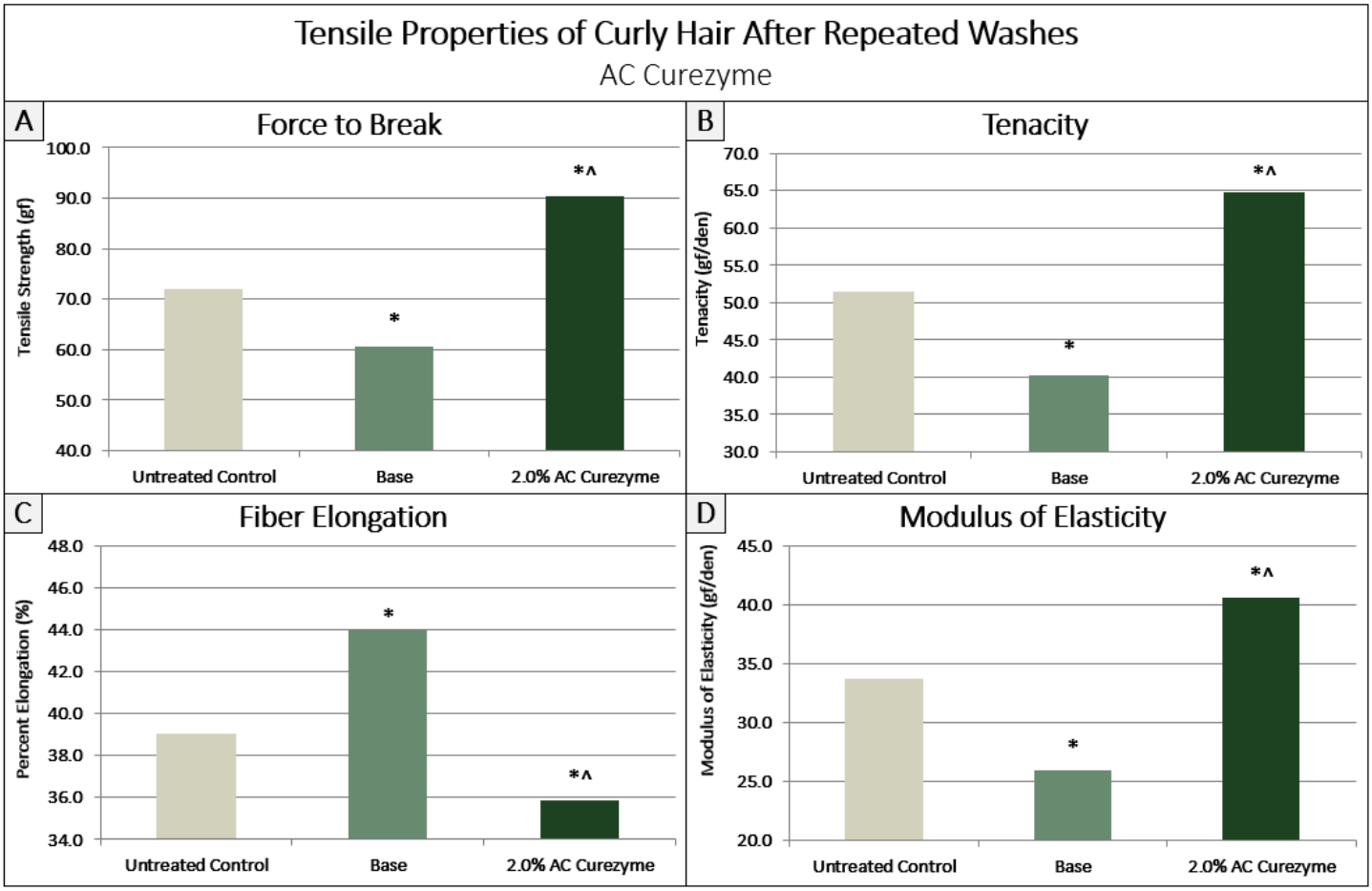

Figure 2. Average Tensile Properties of 3B-Curly-Black Hair Tresses After 14 Washes. * indicates significance ($p \leq 0.05$) compared to the Untreated Control. ^ indicates significance ($p \leq 0.05$) compared to the Base Shampoo and Conditioner (Base). a) Force to Break gives the maximum force required to break each fiber where an increase indicates fiber protection, and a decrease indicates less protection. b) Tenacity gives the break strength of each fiber where an increase indicates fiber protection, and a decrease indicates less protection. c) Fiber Elongation illustrates fiber deformation before breaking where lower values indicate stronger hair fibers and higher values indicate more damaged fibers. d) Modulus of Elasticity expresses the ratio of change in stress to change in strain as a fraction of the original hair fiber where an increase indicates stronger hair fibers, and a decrease indicates more damaged fibers.

Table 4. P-values from one-way ANOVA between Conditions for 3B-Curly-Black Hair Tresses. * indicates significance ($p \leq 0.05$) compared to Untreated Control. ^ indicates significance ($p \leq 0.05$) compared to Base (Base Shampoo and Conditioner).

		Base	2.0% AC Curezyme
Force to Break	Untreated Control	< 0.05*	< 0.05*
	Base	-----	< 0.05^
Tenacity	Untreated Control	< 0.05*	< 0.05*
	Base	-----	< 0.05^
Fiber Elongation	Untreated Control	< 0.05*	< 0.05*
	Base	-----	< 0.05^
Modulus of Elasticity	Untreated Control	< 0.05*	< 0.05*
	Base	-----	< 0.05^

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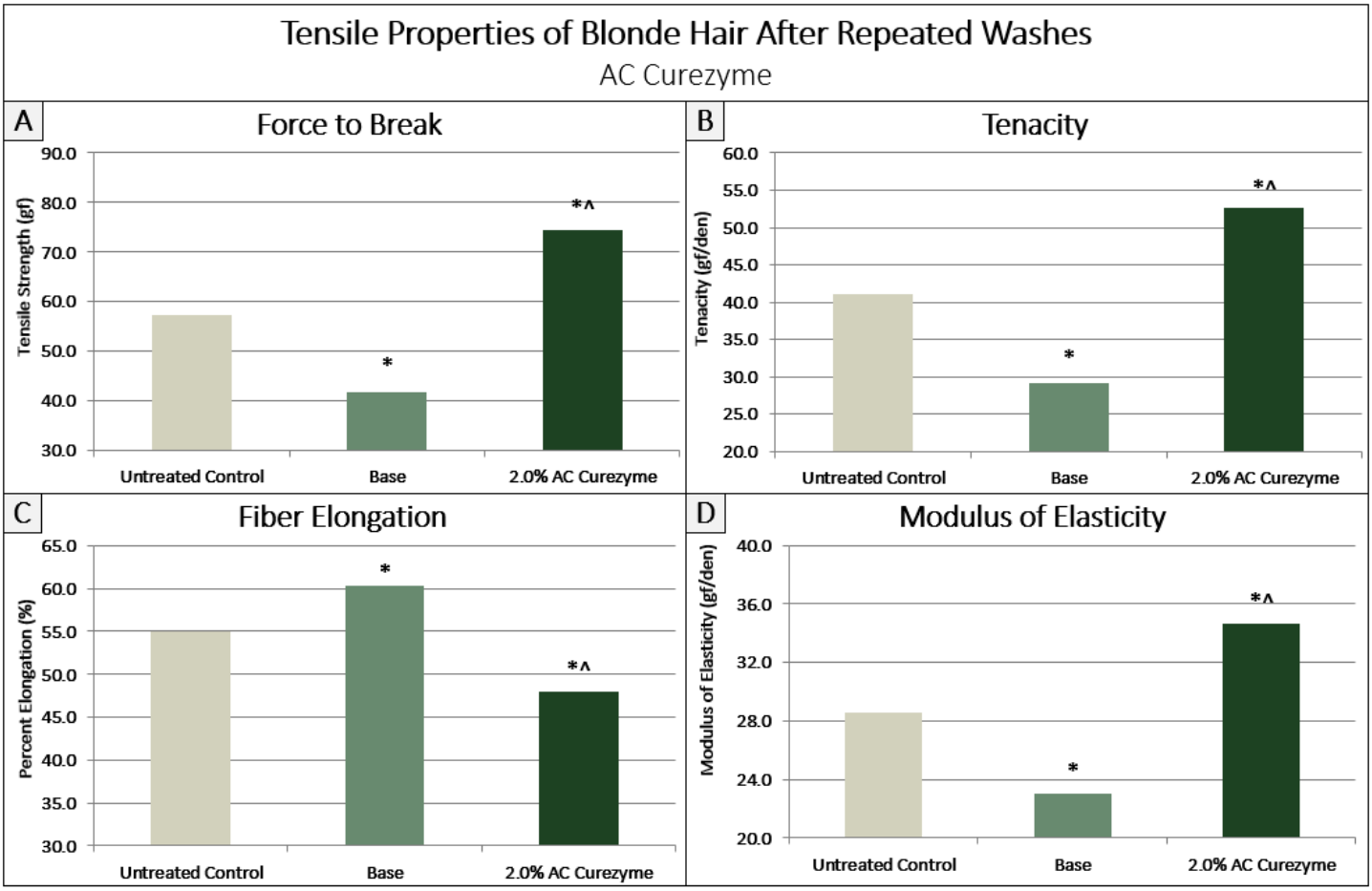

Figure 3. Average Tensile Properties of 1B-Straight-Blonde Hair Tresses After 14 Washes. * indicates significance ($p \leq 0.05$) compared to the Untreated Control. ^ indicates significance ($p \leq 0.05$) compared to the Base Shampoo and Conditioner (Base). a) Force to Break gives the maximum force required to break each fiber where an increase indicates fiber protection, and a decrease indicates less protection. b) Tenacity gives the break strength of each fiber where an increase indicates fiber protection, and a decrease indicates less protection. c) Fiber Elongation illustrates fiber deformation before breaking where lower values indicate stronger hair fibers and higher values indicate more damaged fibers. d) Modulus of Elasticity expresses the ratio of change in stress to change in strain as a fraction of the original hair fiber where an increase indicates stronger hair fibers, and a decrease indicates more damaged fibers.

Table 5. P-values from one-way ANOVA between Conditions for 1B-Straight-Blonde Hair Tresses. * indicates significance ($p \leq 0.05$) compared to Untreated Control. ^ indicates significance ($p \leq 0.05$) compared to Base (Base Shampoo and Conditioner).

		Base	2.0% AC Curezyme
Force to Break	Untreated Control	< 0.05*	< 0.05*
	Base	-----	< 0.05^
Tenacity	Untreated Control	< 0.05*	< 0.05*
	Base	-----	< 0.05^
Fiber Elongation	Untreated Control	< 0.05*	< 0.05*
	Base	-----	< 0.05^
Modulus of Elasticity	Untreated Control	< 0.05*	< 0.05*
	Base	-----	< 0.05^

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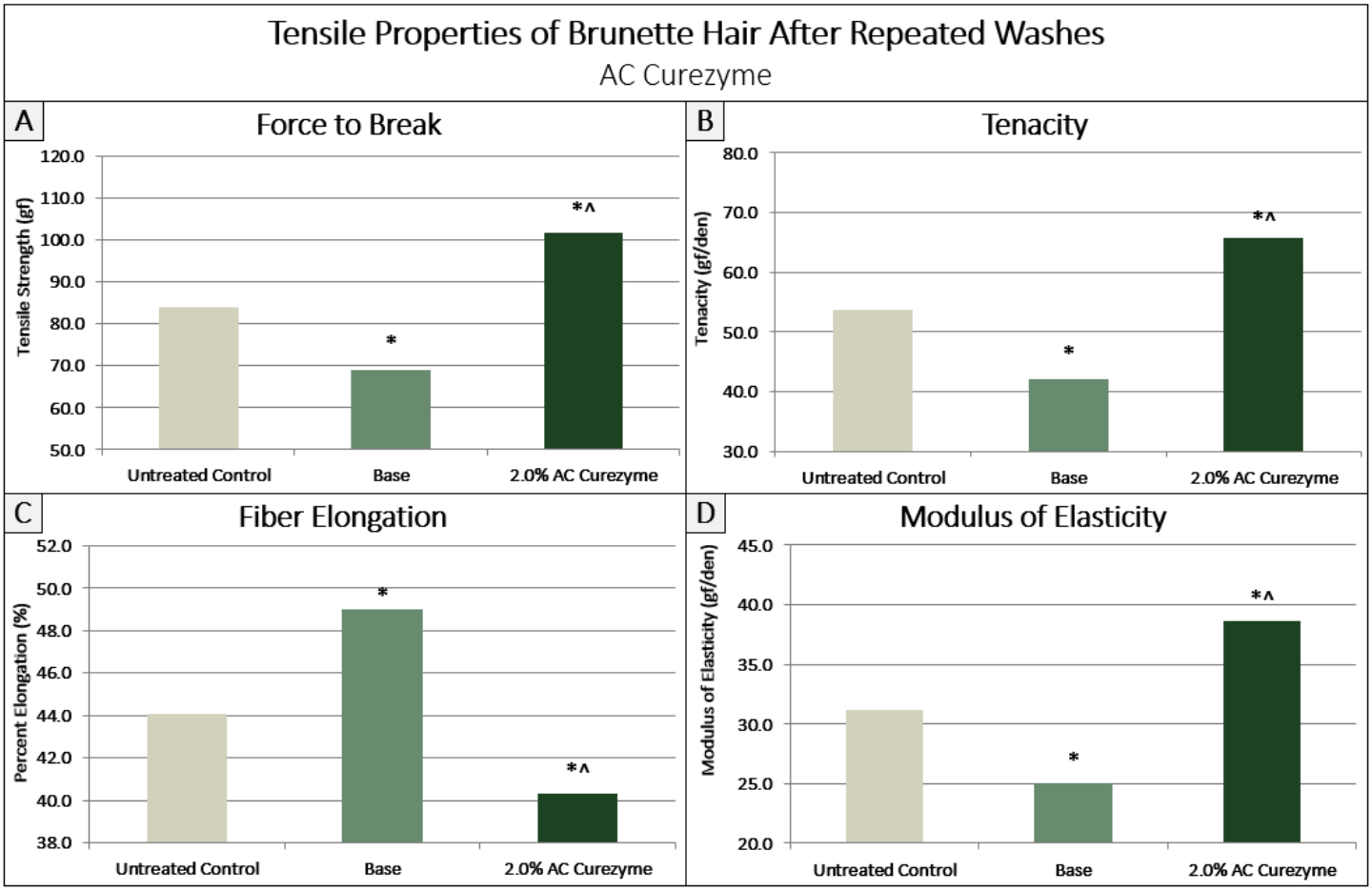

Figure 4. Average Tensile Properties of 2B-Wavy-Brunette Hair Tresses After 14 Washes. * indicates significance ($p \leq 0.05$) compared to the Untreated Control. ^ indicates significance ($p \leq 0.05$) compared to the Base Shampoo and Conditioner (Base). a) Force to Break gives the maximum force required to break each fiber where an increase indicates fiber protection, and a decrease indicates less protection. b) Tenacity gives the break strength of each fiber where an increase indicates fiber protection, and a decrease indicates less protection. c) Fiber Elongation illustrates fiber deformation before breaking where lower values indicate stronger hair fibers and higher values indicate more damaged fibers. d) Modulus of Elasticity expresses the ratio of change in stress to change in strain as a fraction of the original hair fiber where an increase indicates stronger hair fibers, and a decrease indicates more damaged fibers.

Table 6. P-values from one-way ANOVA between Conditions for 2B-Wavy-Brunette Hair Tresses. * indicates significance ($p \leq 0.05$) compared to Untreated Control. ^ indicates significance ($p \leq 0.05$) compared to Base (Base Shampoo and Conditioner).

		Base	2.0% AC Curezyme
Force to Break	Untreated Control	< 0.05*	< 0.05*
	Base	-----	< 0.05^
Tenacity	Untreated Control	< 0.05*	< 0.05*
	Base	-----	< 0.05^
Fiber Elongation	Untreated Control	< 0.05*	< 0.05*
	Base	-----	< 0.05^
Modulus of Elasticity	Untreated Control	< 0.05*	< 0.05*
	Base	-----	< 0.05^

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Discussion

As demonstrated in Figures 1 – 4, repeatedly washing with Base Shampoo and Conditioner negatively impacts the tensile properties of 1C-Straight-Black, 3B-Curly-Black, 1B-Straight-Blonde, and 2B-Wavy-Brunette hair tresses. Together, this data demonstrates repeated hair washing causes significant damage to diverse hair types by degrading the tensile properties of individual fibers.

With respect to 1C-Straight-Black hair, incorporating 2.0% **AC Curezyme** into shampoo and conditioner formulations enhances the tensile properties after repeated washes. Specifically, after 14 washes, 1C-Straight-Black hair tresses washed with the Base Shampoo and Conditioner demonstrated 24%, 6%, and 27% reductions in force to break, tenacity, and elasticity, respectively, and negatively impacted fiber elongation by 7% compared to the Untreated Control (Figure 1; Table 3). Conversely, 2.0% **AC Curezyme** elicited 17%, 22%, and 24% increases in force to break, tenacity, and elasticity, respectively, and improved fiber elongation by 11% after 14 washes in 1C-Straight-Black hair tresses compared to the Untreated Control (Figure 1; Table 3). In 1C-Straight-Black hair tresses, the addition of 2.0% **AC Curezyme** to Base Shampoo and Conditioner improved force to break, tenacity, fiber elongation, and elasticity by 54%, 31%, 17%, and 70% compared to Base Shampoo and Conditioner alone after 14 washes (Figure 1; Table 3). Collectively, this data indicates **AC Curezyme** protects and strengthens 1C-Straight-Black hair after repeated washes.

Regarding 3B-Curly-Black hair, incorporating 2.0% **AC Curezyme** into shampoo and conditioner formulations enhances the tensile properties after repeated washes. Specifically, after 14 washes, curly hair tresses washed with the Base Shampoo and Conditioner demonstrated 16%, 22%, and 23% reductions in force to break, tenacity, and elasticity, respectively, and negatively impacted fiber elongation by 13% compared to the Untreated Control (Figure 2; Table 4). Conversely, 2.0% **AC Curezyme** elicited 26%, 26%, and 21% increases in force to break, tenacity, and elasticity, respectively, and improved fiber elongation by 8% after 14 washes in 3B-Curly-Black hair tresses compared to the Untreated Control (Figure 2; Table 4). In 3B-Curly-Black hair tresses, the addition of 2.0% **AC Curezyme** to Base Shampoo and Conditioner improved force to break, tenacity, fiber elongation, and elasticity by 50%, 61%, 18%, and 56% compared to Base Shampoo and Conditioner alone after 14 washes (Figure 2; Table 4). Together, this data indicates **AC Curezyme** protects and strengthens 3B-Curly-Black hair after repeated washes.

In terms of 1B-Straight-Blonde hair, incorporating 2.0% **AC Curezyme** into shampoo and conditioner formulations enhances the tensile properties after repeated washes. Specifically, after 14 washes, blonde hair tresses washed with the Base Shampoo and Conditioner demonstrated 27%, 29%, and 19% reductions in force to break, tenacity, and elasticity, respectively, and negatively impacted fiber elongation by 10% compared to the Untreated Control (Figure 3; Table 5). Conversely, 2.0% **AC Curezyme** elicited 30%, 29%, and 21% increases in force to break, tenacity, and elasticity, respectively, and improved fiber elongation by 13% after 14 washes in 1B-Straight-Blonde hair tresses compared to the Untreated Control (Figure 3; Table 5). In 1B-Straight-Blonde hair tresses, the addition of 2.0% **AC Curezyme** to Base Shampoo and Conditioner improved force to break, tenacity, fiber elongation, and elasticity by 78%, 81%, 20%, and 51% compared to Base Shampoo and Conditioner alone after 14 washes (Figure 3; Table 5). Together, this data indicates **AC Curezyme** protects and strengthens 1B-Straight-Blonde hair after repeated washes.

Lastly, incorporating 2.0% **AC Curezyme** into shampoo and conditioner formulations enhances the tensile properties of 2B-Wavy-Brunette hair after repeated washes. Specifically, after 14 washes, 2B-Wavy-Brunette hair tresses washed with the Base Shampoo and Conditioner demonstrated 18%, 22%, and 20% reductions in force to break, tenacity, and elasticity, respectively, and negatively impacted fiber elongation by 11% compared to the Untreated Control (Figure 4; Table 6). Conversely, 2.0% **AC Curezyme** elicited 21%, 22%, and 24% increases in force to break, tenacity, and elasticity, respectively, and improved fiber elongation by 8% after 14 washes in 2B-Wavy-Brunette hair tresses compared to the Untreated Control (Figure 4; Table 6). In 2B-Wavy-Brunette hair tresses, the addition of 2.0% **AC Curezyme** to Base Shampoo and Conditioner improved force to break, tenacity, fiber elongation, and elasticity by 48%, 56%, 18%, and 54% compared to Base Shampoo and Conditioner alone after 14 washes (Figure 4; Table 6). Collectively, this data indicates **AC Curezyme** protects and strengthens 2B-Wavy-Brunette hair after repeated washes.

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Taken together, these results demonstrate **AC Curezyme** augments the tensile properties of 1C-Straight-Black, 3B-Curly-Black, 1B-Straight-Blonde, and 2B-Wavy-Brunette hair after repeated washing when added to shampoo and conditioner formulations. In summary, **AC Curezyme** protects and strengthens diverse hair types from the damaging effects of regular washing.