

	Test Article 1	Test Article 2
<u>Tradename:</u>	ACB Kale Protein Blend	AC Wheat Hydrolysate PF
<u>Code:</u>	20036	20615PF
<u>CAS #:</u>	100209-45-8 & 100209-45-8 & 100209-45-8	70084-87-6 (or) 94350-06-8 (or) 73049-73-7
<u>Lot #:</u>	9403183	9404342
<u>Test Request Form #:</u>	11786	

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Test Performed:

Thermal Protection Assay

Introduction

Heat styling, chemical coloring, and other environmental aggressors lead to extensive damage of hair fibers causing thinning, split ends, and breakage. Ceramic straighteners and other heat styling appliances operate at temperatures up to 450°F which causes significant damage to hair keratin and structure. Protecting hair from these high temperatures is vital in preventing dry, brittle ends as well as permanent damage. Application of cosmetic products before heat styling helps combat the deleterious effects by strengthening and protecting hair fibers. Therefore, products designed to maintain the structural integrity of the hair cuticle and reduce the damage caused by heat styling are essential.

Physical strength of hair can be measured using tensile properties such as tenacity and elasticity. Tenacity reveals the breaking strength of the hair where the higher force needed indicates a stronger and healthier fiber. Force is applied to the hair strands individually until each strand breaks. The Modulus of Elasticity identifies the resistance to being deformed and provides the strength needed to increase fiber length. Force is applied on the hair causing elongation and elasticity is expressed as the ratio of change in stress to change in strain as a fraction of the original hair fiber length. The greater the stress needed the more elastic and stronger the hair. A product that provides thermal protection will maintain hair tenacity and elasticity similar to untreated hair.

Accordingly, a thermal protection assay was conducted to assess the ability of **ACB Kale Protein Blend** to protect hair against heat styling. Additionally, **AC Wheat Hydrolysate PF** was tested to provide comparative data to a well-known plant protein.

Assay Principle

Virgin and chemically treated human hair tresses were tested to understand the protective capability of a cosmetic product. Heat styling was conducted using a ceramic straightener on virgin and chemically processed hair tresses. Tresses were treated with testing materials before exposure to heat styling. After styling, the physical properties of individual hair fibers were assessed utilizing the Instron which measures tensile properties of hair.

Materials

- A. **Hair Samples:** Human Virgin Brunette and Bleached Blonde Hair Tresses
- B. **Products:** Base Shampoo and Conditioner (Table 1); Deionized Water (DI H₂O)
- C. **Equipment:** Remington Ceramic Hair Straightener; Instron (Method ASTM D3822)
- D. **Software:** Excel Analysis ToolPak (Microsoft)
- E. **Other:** Plastic Syringes

Table 1. Base Shampoo and Base Conditioner Compositional Breakdowns.

Base Shampoo Formulation		Base Conditioner Formulation	
INCI	%	INCI	%
Water	41.0	Water	76.0
Guar Hydroxypropyltrimonium Chloride	1.0	Polyquaternium-10	1.0
Sodium Methyl 2-Sulfolaurate (and) Disodium 2-Sulfolaurate	35.0	Glycerin	3.0
Cocamidopropyl Betaine	15.0	Water & Centrimonium Chloride	2.0
Lactobacillus Ferment & Lactobacillus & Cocos Nucifera (Coconut) Fruit Extract	4.0	Behentrimonium Methosulfate & Cetearyl Alcohol & Butylene Glycol	8.0
Polysorbate 20	2.0	Hydrogenated Ethylhexyl Olivat (and) Hydrogenated Olive Oil Unsaponifiables	5.0
Fragrance	2.0	Lactobacillus Ferment	4.0
		Fragrance	1.0

Methods

Ten hair tresses (five virgin brunette and five bleach blonde) were collected and assigned to each condition described in Table 2. Eight tresses were washed once with Base Shampoo and Conditioner and allowed to fully air dry before heat styling. While damp, two experimental tresses received 0.25 mL of 2.0% **ACB Kale Protein Blend** in Deionized Water (DI H₂O) and two received 0.25 mL of 2.0% **AC Wheat Hydrolysate PF** in DI H₂O as a leave-in applications. Untreated Control tresses were left unwashed. The Base Control, 2.0% **ACB Kale Protein Blend**, and 2.0% **AC Wheat Hydrolysate PF** treated tresses were heat styled after the final wash using 20 passes of a hair straightener set to the highest setting of 450°F. The Untreated Control and Wash Control tresses did not receive heat styling. The study was conducted using a blind protocol.

Table 2. Descriptions of the Conditions and Treatments for each Hair Tress within each Hair Type.

Condition	Treatment Description
Untreated Control	No Wash(es), No Heat Styling
Wash Control	Wash(es), No Heat Styling
Base Control	Wash(es), Heat Styling
2.0% ACB Kale Protein Blend	Wash(es), Leave-In Application, Heat Styling
2.0% AC Wheat Hydrolysate PF	Wash(es), Leave-In Application, Heat Styling

The Intron method ASTM D3822 was used to evaluate the effect of **ACB Kale Protein Blend** and **AC Wheat Hydrolysate PF** on the integral structure and properties of the hair after heat styling. Single fiber testing was performed to assess the strength of the hair in terms of tenacity and 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 from this study met criteria for a valid assay and the controls performed as anticipated. Compared to the Untreated Control, the Wash Control experienced little to no changes in tenacity and elasticity while the Base Control experienced reduced tenacity and elasticity. Alternatively, the tresses treated with 2.0% **ACB Kale Protein Blend** and 2.0% **AC Wheat Hydrolysate PF** exhibited fortified structural integrity through increased tenacity and elasticity compared to the Base Control. Tresses treated with **AC Kale Protein Blend** provided comparable results to **AC Wheat Hydrolysate PF**.

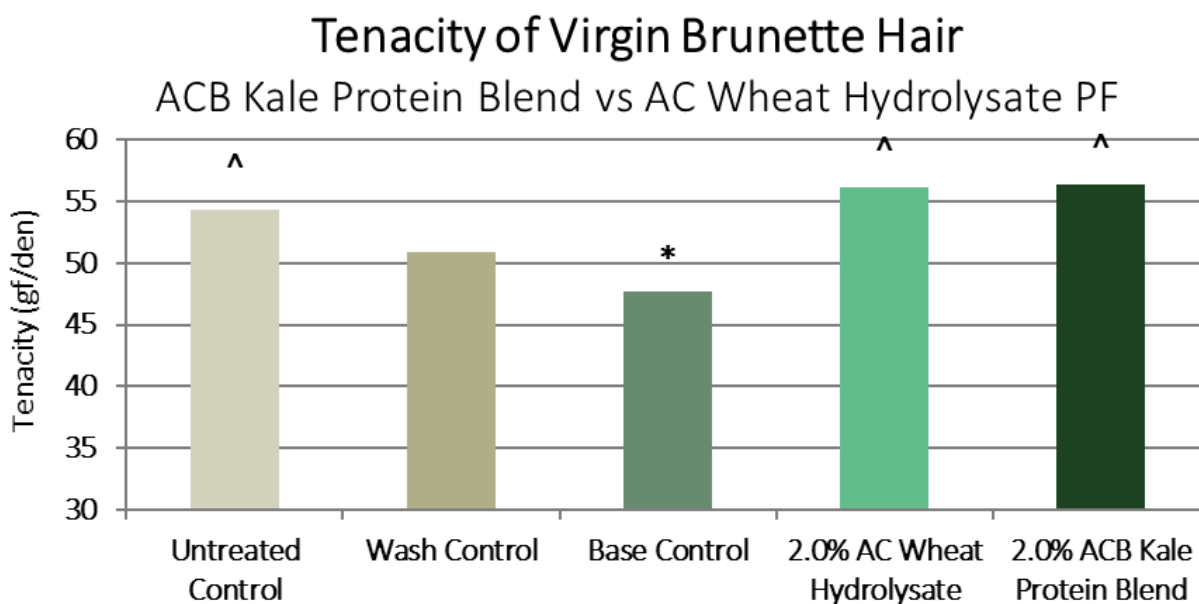


Figure 1. Average Tenacity of Virgin Brunette Hair Tresses. * indicates significance ($p \leq 0.05$) compared to the Untreated Control. ^ indicates significance ($p \leq 0.05$) compared to the Base Control. There is no significant difference in brunette hair tenacity between **AC Wheat Hydrolysate** and **ACB Kale Protein Blend**.

Table 3. Results from one-way ANOVA between Conditions Compared for Virgin Brunette Hair Tenacity (gf/den). * indicates significance ($p \leq 0.05$) compared to the Untreated Control. ^ indicates significance ($p \leq 0.05$) compared to the Base Control.

	Untreated Control	Wash Control	Base Control	2.0% AC Wheat Hydrolysate PF	2.0% ACB Kale Protein Blend
Untreated Control	-----	> 0.05	0.025*	> 0.05	> 0.05
Base Control	0.025^	> 0.05	-----	0.015^	0.039^

Modulus of Elasticity of Virgin Brunette Hair ACB Kale Protein Blend vs AC Wheat Hydrolysate PF

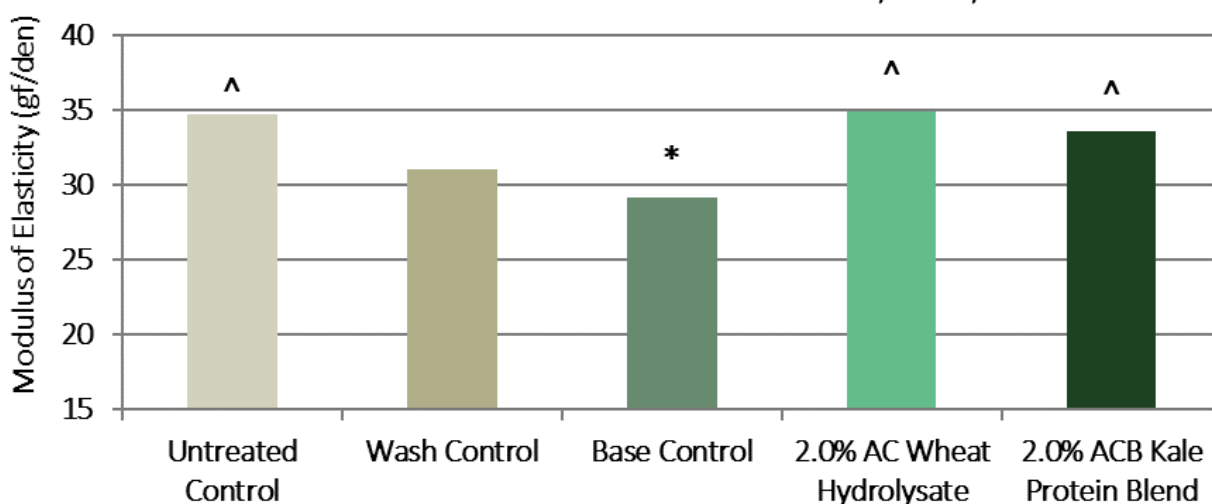


Figure 2. Average Modulus of Elasticity of Virgin Brunette Hair. * indicates significance ($p \leq 0.05$) compared to the Untreated Control. ^ indicates significance ($p \leq 0.05$) compared to the Base Control. There is no significant difference in brunette hair modulus of elasticity between **AC Wheat Hydrolysate** and **ACB Kale Protein Blend**.

Table 4. Results from one-way ANOVA between Conditions Compared for Virgin Brunette Modulus of Elasticity (gf/den). * indicates significance ($p \leq 0.05$) compared to the Untreated Control. ^ indicates significance ($p \leq 0.05$) compared to the Base Control.

	Untreated Control	Wash Control	Base Control	2.0% AC Wheat Hydrolysate PF	2.0% ACB Kale Protein Blend
Untreated Control	-----	> 0.05	0.036*	> 0.05	> 0.05
Base Control	0.036^	> 0.05	-----	0.040^	0.015^

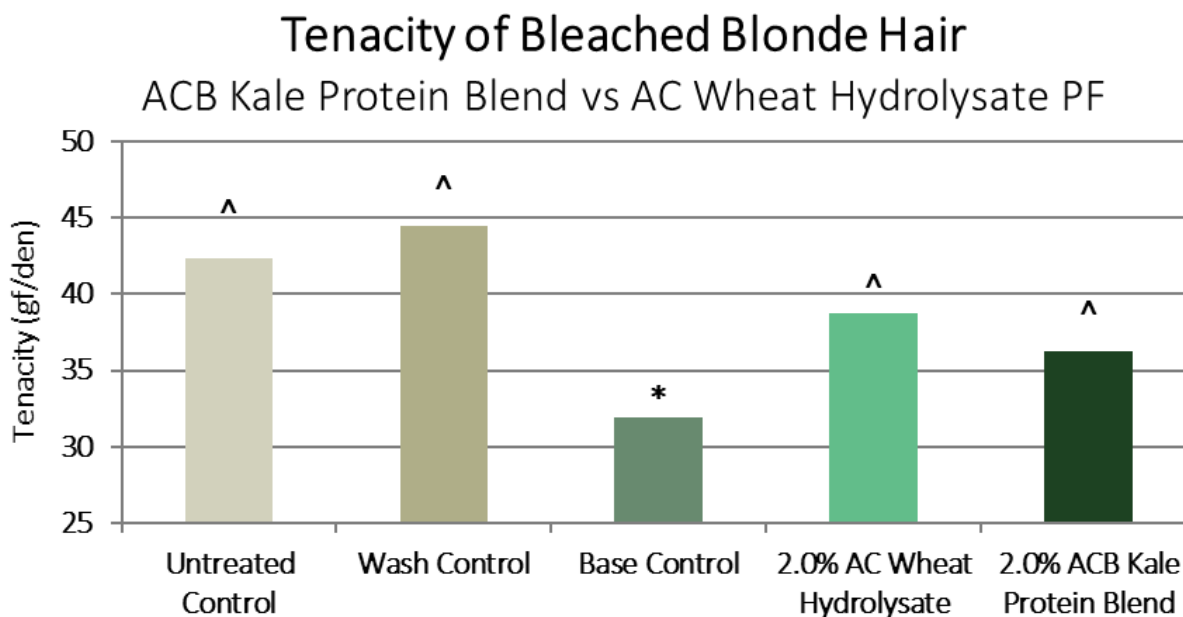


Figure 3. Average Tenacity of Bleached Blonde Hair Tresses. * indicates significance ($p \leq 0.05$) compared to the Untreated Control. ^ indicates significance ($p \leq 0.05$) compared to the Base Control. There is no significant difference in bleached blonde hair tenacity between **AC Wheat Hydrolysate** and **ACB Kale Protein Blend**.

Table 5. Results from one-way ANOVA between Conditions Compared for Bleached Blonde Hair Tenacity (gf/den). * indicates significance ($p \leq 0.05$) compared to the Untreated Control. ^ indicates significance ($p \leq 0.05$) compared to the Base Control.

	Untreated Control	Wash Control	Base Control	2.0% AC Wheat Hydrolysate PF	2.0% ACB Kale Protein Blend
Untreated Control	-----	> 0.05	0.017*	> 0.05	> 0.05
Base Control	0.017^	0.005^	-----	0.032^	0.025^

Modulus of Elasticity of Bleached Blonde Hair ACB Kale Protein Blend vs AC Wheat Hydrolysate PF

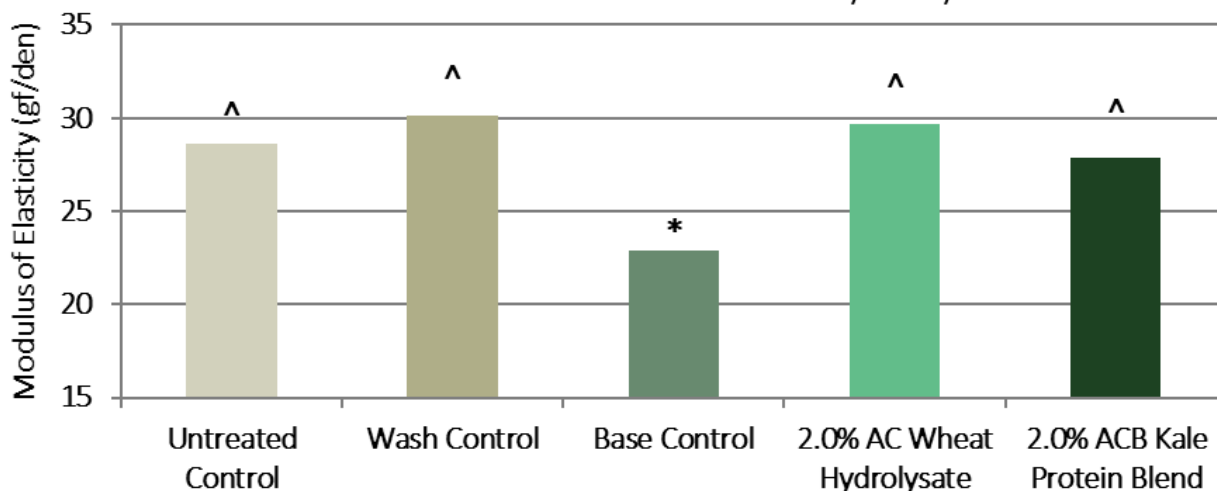


Figure 4. Average Modulus of Elasticity of Bleached Blonde Hair. * indicates significance ($p \leq 0.05$) compared to the Untreated Control. ^ indicates significance ($p \leq 0.05$) compared to the Base Control. There is no significant difference in bleached blonde hair modulus of elasticity between **AC Wheat Hydrolysate** and **ACB Kale Protein Blend**.

Table 6. Results from one-way ANOVA between Conditions Compared for Bleached Blonde Modulus of Elasticity (gf/den). * indicates significance ($p \leq 0.05$) compared to the Untreated Control. ^ indicates significance ($p \leq 0.05$) compared to the Base Control.

	Untreated Control	Wash Control	Base Control	2.0% AC Wheat Hydrolysate PF	2.0% ACB Kale Protein Blend
Untreated Control	-----	> 0.05	0.038*	> 0.05	> 0.05
Base Control	0.038^	0.012^	-----	0.030^	0.038^

Discussion

A thermal protection assay was performed to determine the protective benefits of **ACB Kale Protein Blend** on hair with respect to heat styling. Additionally, **2.0% AC Wheat Hydrolysate PF** was tested to provide comparative data between the two plant proteins. The results from this study indicate that both **2.0% ACB Kale Protein Blend** and **2.0% AC Wheat Hydrolysate PF** provide protection and strengthening benefits against standard thermal styling and do so comparably.

As shown in Figures 1 and 2, the virgin brunette hair utilized as the Untreated Control exhibited an average tenacity of 54 gf/den and an average modulus of elasticity of 35 gf/den. The virgin brunette hair treated as the Wash Control experienced insignificant decreases of 6% and 11% in tenacity and modulus of elasticity, respectively, compared to the Untreated Control (Tables 3 and 4). This data indicates washing alone does not significantly reduce virgin brunette hair strength. Conversely, the virgin brunette hair treated as the Base Control exhibited significant reductions in tenacity and modulus of elasticity of 12% and 16%, respectively, compared to the Untreated Control confirming the damaging effects of heat styling on virgin brunette hair. **AC Wheat Hydrolysate** provided protection to virgin brunette hair tresses via increased tenacity of 3% and the modulus of elasticity remained unchanged compared to the Untreated Control. Finally, application of **ACB Kale Protein Blend** on virgin brunette hair tresses increased hair tenacity by 4% and only experienced a 3% decrease in modulus of elasticity compared to the Untreated Control. Both **AC Wheat Hydrolysate** and **ACB Kale Protein Blend** significantly protected tenacity and modulus of elasticity compared to the Base Control alone. Moreover, there was no significant difference between **AC Wheat Hydrolysate** and **ACB Kale Protein Blend** with respect to tenacity or modulus of elasticity indicating the products provide equivalent protection to virgin brunette hair. These data indicate applying **ACB Kale Protein Blend** protects and strengthens virgin brunette hair against thermal damage.

The bleached blonde hair utilized as the Untreated Control exhibited an average tenacity of 42 gf/den and an average modulus of elasticity of 29 gf/den (Figures 3 and 4). The bleached blonde hair treated as the Wash Control experienced insignificant increases of 5% and 5% in tenacity and modulus of elasticity, respectively, compared to the Untreated Control (Tables 5 and 6). This data indicates washing alone does not significantly impact chemically treated hair strength. Conversely, the bleached blonde hair treated as the Base Control exhibited significant reductions in tenacity and modulus of elasticity of 25% and 20%, respectively, compared to the Untreated Control confirming the damaging effects of heat styling on chemically treated hair. **AC Wheat Hydrolysate** provided protection to bleached blonde hair tresses via an insignificant decrease in tenacity of 8% and an increase of 4% in modulus of elasticity compared to the Untreated Control. Finally, application of **ACB Kale Protein Blend** on bleached blonde hair tresses provided protection with insignificant reductions in hair tenacity of 14% and in modulus of elasticity of 3% compared to the Untreated Control. Both **AC Wheat Hydrolysate** and **ACB Kale Protein Blend** significantly protected tenacity and modulus of elasticity compared to the Base Control alone. Moreover, there was no significant difference between **AC Wheat Hydrolysate** and **ACB Kale Protein Blend** with respect to tenacity or modulus of elasticity indicating the products provide equivalent protection to bleached blonde hair. These data indicate applying **ACB Kale Protein Blend** protects and strengthens bleached blonde hair against thermal damage.

Collectively, heat styling damages virgin brunette and bleached blonde hair, however one application of **ACB Kale Protein Blend** prevents the heat-styled induced reductions in tenacity and modulus of elasticity in both hair types when utilized as a leave-in product. Importantly, the results from **ACB Kale Protein Blend** are comparable to a more commonly used plant protein **AC Wheat Hydrolysate PF**. Taken together, **ACB Kale Protein Blend** protects and strengthens virgin brunette and bleached blonde hair against thermal damage.