ACB Pisum Sativum Peptide
Efficacy Data

<table>
<thead>
<tr>
<th>Name of Study</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hair Hydration Assay</td>
<td>Increased hair hydration is a key benefit of hydrolyzed proteins. The results indicated a comparable increase in hair hydration on subjects using both a 5.0% solution of ACB Pisum Sativum Peptide and a 5.0% solution of Wheat Hydrolysate.</td>
</tr>
<tr>
<td>Volumizing Assay</td>
<td>This study measured the diameter of color treated hair at different intervals to determine an increase in hair thickness. A 2.0% solution of ACB Pisum Sativum Peptide or 2.0% solution of Wheat Hydrolysate was applied to each strand of test hair. Immediate results showed strands treated with ACB Pisum Sativum Peptide had an average increase in hair diameter of 14.08% with an average increase of 13.65% one hour after application, in comparison to the strands treated with Wheat Hydrolysate, which experienced no increase in diameter initially or over time.</td>
</tr>
<tr>
<td>Salon Half-Head Study</td>
<td>ACB Pisum Sativum Peptide is great for improved hydration and shine of the hair. It was also reported that this product helped enhance volume and did not weigh hair down. Perfect for use in treatments to enhance shine and hydration for healthier hair.</td>
</tr>
<tr>
<td>ORAC Assay</td>
<td>ACB Pisum Sativum Peptide exhibited potent antioxidant activity comparable to 100μM Trolox®. The antioxidant capacity of ACB Pisum Sativum Peptide increased as the concentration increased, as a result</td>
</tr>
</tbody>
</table>

Code: 16810
INCI Name: Pisum Sativum (Pea) Peptide
CAS #: 90082-41-0
EINECS #: 290-130-6
we can assure that its ability to minimize oxidative stress is dose dependent.

<table>
<thead>
<tr>
<th>Sirius Red Fast Green Report Analysis</th>
</tr>
</thead>
<tbody>
<tr>
<td>ACB Pism Sativum Peptide exhibited positive collagen synthesis activity. The increase in collagen production may lead to improvement in the dermal-epidermal junction integrity as well as an improved scaffolding matrix. For these reasons, we can assume ACB Pism Sativum Peptide is suitable for cosmetic applications designed to boost collagen synthesis to aid in providing a younger and healthier complexion.</td>
</tr>
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</table>
Hair Hydration Comparison Assay

Tradename: ACB Pisum Sativum Peptide

Code: 16810

Lot #: NC180315-F

CAS #: 100209-45-8

Test Request Form #: 4642

Sponsor: Active Concepts, LLC; 107 Technology Drive Lincolnton, NC 28092
Study Director: Maureen Danaher
Principle Investigator: Parisa Mehrzadeh

Test Performed: Gravimetric Analysis of Hair Hydration

Introduction
The study was conducted to evaluate the hair hydration benefits of ACB Pisum Sativum Peptide by gravimetric means.

Materials
A. Equipment: Sealed glass chamber, Relative humidity monitor, Analytical balance (Mettler Toledo Model ME4002E). This study was conducted using Sensationnel Bare & Natural Brazilian 100% Virgin Remi Unprocessed Human Hair (Hair Zone Moonachie, NJ).

Methods
Gravimetric analysis is an analytical method in which the analytical signal is a measurement of mass or a change in mass. Substantivity of a material can be measured as a change in mass after the material is exposed to controlled humidity. An increase in hydration can be measured by comparing the weight of the test material at over time after application and signifies hydrating capabilities.

Before measuring the moisturizing effect, the hair swatches were kept in a humidity controlled box (22°C, 50% Relative Humidity) for 24 hours. Hair swatches were weighed on an analytical balance and their starting weight was recorded. The hair swatches were immersed in either 5.0% ACB Pisum Sativum Peptide aqueous solution, 5.0% Wheat Hydrolysate aqueous solution (positive control) or left untreated (negative control). The treated swatches were immersed in their respective solutions for three hours at 22°C and then rinsed with deionized water. The hair swatches were air dried in the humidity controlled box (22°C, 50% Relative Humidity) for 48 hours. The swatches were then weighed and with the analytical balance for final measurement.
Results

**Figure 1.** Average Percent Increase in Hydration.

**Discussion**

Both the innovative hydrolysis method used in the production of **ACB Pisum Sativum Peptide** and the traditional method of hydrolysis induced when creating wheat hydrolysate yield a protein hydrolysate capable of producing hair hydrating benefits at virtually identical levels. This demonstrates a novel approach in hydrolysis, such as the one used in the manufacturing of **ACB Pisum Sativum Peptide** provides just as much moisture as hydrolyzed wheat protein with proven hydration benefits.
Abstract

Hydrolyzed proteins, such as Oat, Soy and Wheat have been used in hair care as a traditional means to hydrate the hair and provide strengthening properties. Until recently, hydrolysis was induced using acid, water, or fermentation. Active Concepts has implemented an innovative hydrolysis approach to the newest and most bio-available vegetable protein on the market, Pisum Sativum Peptide. This microorganism prompted hydrolysis creates the by product, lactic acid, as a secretion which provides volumizing and anti-aging benefits measured.

Materials and Methods

The hair samples used in this study were tested using identical intervals and percentages of two protein hydrolysates, ACB Pisum Sativum Peptide and Wheat Hydrolysate. The materials used in the procedure to determine the diameter of each strand were an untreated control hair sample, the control hair sample (treated with 2.0% Wheat Hydrolysate Solution), and the sample treated with the test material (2.0% ACB Pisum Sativum Peptide). Each hair was imaged and measured before a solution was applied. The hairs were then removed from the slide and either placed in the 2.0% solution of the Wheat Hydrolysate or the 2.0% solution of ACB Pisum Sativum Peptide. Each hair was removed, measured and imaged then placed aside. After four hours, each hair was reimaged and measured to demonstrate sustained volume potential of each respective hydrolyzed protein.
Results

Effects on Hair Diameter

![Graph showing percent increase in hair diameter over time after treatment with ACB Pisum Sativum Peptide and Wheat Hydrolysate.](image)

**Figure 1.** Percent increase in hair diameter over time after treatment with ACB Pisum Sativum Peptide and Wheat Hydrolysate.

**Microscopy Imaging of Individual Hair Strands**

**Figure 2:** Individual strand immediately following treatment with wheat Hydrolysate (note beading).

**Figure 3:** Individual strand immediately following treatment with ACB Pisum Sativum Peptide.
Discussion

Immediate results showed an average increase in hair diameter of 14.08% with an average increase of 13.65% four hours following the initial application. After placing individual hair strands under a microscope, Microscopy Imaging of the individual strands were taken to visually demonstrate the increase in hair diameter achieved when using **ACB Pisum Sativum Peptide** at 2.0% in a solution compared to the use of Wheat Hydrolysate at 2.0% in a solution. **ACB Pisum Sativum Peptide** is able to effectively volumize the hair for thicker and younger looking hair, a revolutionary step for anti-aging hair care products.
Salon Half-Head Study

**ABSTRACT**

The condition of the cuticle (the outer most layer of the hair) significantly affects both the manageability and sleekness of our hair. Over time, hair can become damaged, which can result in the cuticle lifting because of both environmental and styling influences and processes. The result: lifeless, dull hair that is difficult to manage. Improving the sleekness of hair has been shown to instantly create a healthier more youthful appearance. Increasing combability not only eases manageability, but also helps to minimize physical damage that perpetuates the loss of body and difficulty in styling.

**ACB Pisum Sativum Peptide** is a product designed to increase the volume of the hair while providing hydration and antioxidant properties for protection against stressors. However this unique ingredient also enhances shine, dry and wet combability, manageability and the smoothness of the hair. The purpose of this study was to confirm whether or not **ACB Pisum Sativum Peptide** is capable of providing these additional benefits in a shampoo and conditioner application.

A half head study was conducted to determine the comparison of a control shampoo vs. 2.0% **ACB Pisum Sativum Peptide** in the control shampoo. Additionally, a comparison between the control conditioner and 2.0% **ACB Pisum Sativum Peptide** in the control conditioner were reported. Each volunteer’s hair was photographed prior to the treatment and again after the shampoo and conditioner had been applied and the hair was styled. The images of the half head study were used in conjunction with a sensory assessment subjectively rating the parameters - cleansing, smoothing, dry and wet combability, anti-frizz, overall feel, shine and hydration. This assessment was conducted both before and after treatment. Based on the results obtained, **ACB Pisum Sativum Peptide** is capable of enhancing wet and dry combability, anti-frizz, overall feel, shine and hydration of the hair. These attributes make it an ideal ingredient for use in products intended for all hair types.
Salon Half-Head Study

MATERIALS AND METHODS

The study was conducted using five participants. Each subject had their baseline photo taken prior to having their hair washed. The participant was also asked to complete a survey rating their hair prior to treatment on a scale of 1 to 10, with 1 being the lowest and 10 being the highest, using the following parameters cleansing, smoothing, dry and wet combability, anti-frizz, overall feel, shine and hydration.

Half of the head was treated with the control shampoo and conditioner while the other half of the head was treated with 2.0% ACB Pisum Sativum Peptide in the base shampoo and base conditioner. After the application and rinse of the test and positive control products, each participant’s hair was blown dry using a round brush on both sides of the head. Once the hair was completely dry, the participant was asked to again assess the same parameters of both halves of their hair. Assessments were made using a rubric from 1 to 10, with 1 being the lowest and 10 being the highest.

RESULTS

<table>
<thead>
<tr>
<th>Parameters Tested</th>
<th>Assessment of the Control Shampoo</th>
<th>Assessment of the Experimental (2.0% ACB Pisum Sativum Peptide in Control Shampoo)</th>
<th>Assessment of the Control Conditioner</th>
<th>Assessment of the Experimental (2.0% ACB Pisum Sativum Peptide in Control Conditioner)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cleansing</td>
<td>6.25</td>
<td>7.25</td>
<td>x</td>
<td>x</td>
</tr>
<tr>
<td>Smoothing</td>
<td>6.14</td>
<td>8.33</td>
<td>6.33</td>
<td>9.25</td>
</tr>
<tr>
<td>Wet Combability</td>
<td>3.33</td>
<td>5.25</td>
<td>6.00</td>
<td>9.15</td>
</tr>
<tr>
<td>Dry Combability</td>
<td>X</td>
<td>X</td>
<td>6.10</td>
<td>8.33</td>
</tr>
<tr>
<td>Anti-Frizz</td>
<td>X</td>
<td>X</td>
<td>5.22</td>
<td>8.46</td>
</tr>
<tr>
<td>Overall Feel</td>
<td>X</td>
<td>X</td>
<td>7.00</td>
<td>9.50</td>
</tr>
<tr>
<td>Shine</td>
<td>X</td>
<td>X</td>
<td>7.50</td>
<td>9.82</td>
</tr>
<tr>
<td>Hydration</td>
<td>X</td>
<td>X</td>
<td>8.25</td>
<td>9.70</td>
</tr>
<tr>
<td>Mean</td>
<td>5.24</td>
<td>6.94</td>
<td>6.165</td>
<td>9.2</td>
</tr>
</tbody>
</table>

Chart 1. Average Results for Participant’s Sensory Assessment.

<table>
<thead>
<tr>
<th>Parameters Tested</th>
<th>Percent Difference – Comparison of Control Shampoo vs. Experimental (2.0% ACB Pisum Sativum Peptide in Control Shampoo)</th>
<th>Percent Difference – Comparison of Control Conditioner vs. Experimental (2.0% ACB Pisum Sativum Peptide in Control Conditioner)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cleansing</td>
<td>14.81%</td>
<td>x</td>
</tr>
<tr>
<td>Smoothing</td>
<td>30.26%</td>
<td>37.48%</td>
</tr>
<tr>
<td>Wet Combability</td>
<td>44.75%</td>
<td>41.58%</td>
</tr>
<tr>
<td>Dry Combability</td>
<td>x</td>
<td>30.90%</td>
</tr>
<tr>
<td>Anti-Frizz</td>
<td>x</td>
<td>47.36%</td>
</tr>
<tr>
<td>Overall Feel</td>
<td>x</td>
<td>30.30%</td>
</tr>
<tr>
<td>Shine</td>
<td>x</td>
<td>26.70%</td>
</tr>
<tr>
<td>Hydration</td>
<td>x</td>
<td>16.15%</td>
</tr>
</tbody>
</table>

Chart 2. Percent Difference of Participant’s Sensory Assessment.
Salon Half-Head Study

Graph 1. Rating of hair characteristics following sensory assessment.

Figure 1. Full head Baseline, Untreated Hair.

Figure 2. Half Head Treated.

Figure 3. Full head Baseline, Untreated Hair.

Figure 4. Half Head Treated.
Salon Half-Head Study

Figure 5. Full head Baseline, Untreated Hair.

Figure 6. Half Head Treated.

Half Head Treated with Control Shampoo and Conditioner

Half-Head Treated with 2.0% ACB Pisum Sativum Peptide in a Base Shampoo & Conditioner

Figure 7. Full head Baseline, Untreated Hair.

Figure 8. Half Head Treated.

Half Head Treated with Control Shampoo and Conditioner

Half-Head Treated with 2.0% ACB Pisum Sativum Peptide in a Base Shampoo & Conditioner

Figure 9. Full head Baseline, Untreated Hair.

Figure 10. Half Head Treated.

Half Head Treated with Control Shampoo and Conditioner

Half-Head Treated with 2.0% ACB Pisum Sativum Peptide in a Base Shampoo & Conditioner
Salon Half-Head Study

When comparing hair characteristics of the baseline assessments to the post style assessments, the benefits of including 2.0% ACB Pisum Sativum Peptide in a shampoo and conditioner are even more apparent. In relation to the baseline readings, the test-half of the head treated with conditioner improved the intended subjective parameters, improving smoothing, wet and dry combability, anti-frizz, overall feel, shine and hydration by 37.48%, 41.58%, 30.90%, 47.36%, 30.30%, 26.70% and 16.15%, respectively. It is clear from the images in this study that ACB Pisum Sativum Peptide helps create a smooth, sleek hairstyle. Additionally, in all images, the hair is noticeably shinier and has a more conditioned appearance.

The professional stylist who performed the actual tests by applying the product, styling the hair and documenting the images said ACB Pisum Sativum Peptide is great for improved hydration and shine of the hair. It was also reported that this product helped enhance volume and did not weigh hair down. Perfect for use in treatments to enhance shine and hydration for healthier hair.

DISCUSSION

The results of the assessment indicate that when incorporated into a shampoo, 2.0% ACB Pisum Sativum Peptide did show improvement in cleansing. However, when used in a conditioner ACB Pisum Sativum Peptide is capable of improving smoothing, dry and wet combability, anti-frizz, overall feel, shine and hydration more than the control conditioner. These results can be further supported by Figures 1 through 10, where clearly the half of the subject’s head treated with 2.0% ACB Pisum Sativum Peptide appears healthy and silky smooth. Additionally, the subjects reported an increase in volume and overall feel of the hair.

Graph 2. Hair Assessment results for sensory characteristics.
Oxygen Radical Absorbance Capacity (ORAC) Assay

**Tradename:** ACB Pisum Sativum Peptide

**Code:** 16810

**CAS #:** 90082-41-0

**Test Request Form #:** 33

**Sponsor:** Active Concepts, LLC; 107 Technology Drive Lincolnton, NC 28092

**Study Director:** Erica Segura

**Principal Investigator:** Meghan Darley

**Test Performed:**
Oxygen Radical Absorbance Capacity (ORAC) Assay

**Introduction**

Reactive oxygen species (ROS) are generated by normal cellular processes, environmental stresses, and UV irradiation. ROS are dangerous to cellular structures and functional molecules (i.e DNA, proteins, lipids) as they act as strong oxidizing agents or free radicals. The oxygen radical absorbance capacity (ORAC) assay is a standard method used to assess antioxidant capacity of physiological fluids, foods, beverages, and natural products. The assay quantitatively measures a sample’s ability to quench free radicals that have the potential to react with and damage cellular components.

Oxygen Radical Absorbance Capacity (ORAC) assay was conducted to assess the antioxidant capacity of **ACB Pisum Sativum Peptide**.

**Assay Principle**

This assay is based upon the effect of peroxyl radicals generated from the thermal decomposition of 2, 2'-azobis-2-methyl-propanimidamide dihydrochloride (AAPH) on the signal intensity from the fluorescent probe, fluorescein, in the presence of an oxygen radical absorbing substance. The degree of change is indicative of the amount of radical damage and the presence of antioxidants results in an inhibition in the free radical damage to the fluorescein. The antioxidant protection of the sample can be calculated by comparing it to a set of known standards. Trolox®, a water soluble vitamin E analog, with known antioxidant capabilities is used in this ORAC assay as the standard for measuring the antioxidant capacity of unknown substances. ORAC values, expressed in µM of Trolox® equivalents (TE), are calculated using the area under the curves (AUC) of the test product, Trolox®, and the control materials. Trolox equivalency is used as the benchmark for antioxidant capacity of mixtures since it is difficult to measure individual components.
Oxygen Radical Absorbance Capacity (ORAC) Assay

Materials

A. Equipment: Synergy H1 Microplate reader (BioTek Instruments, Winooski, VT); Gen5 software (BioTek Instruments, Winooski, VT); Pipettes
B. Buffers: 75mM Potassium Phosphate (pH 7.4); Deionized H2O
C. Reagents: 2,2′-Azobis(2-methylpropionamide) dihydrochloride (AAPH) (153mM); 6-Hydroxy-2,5,7,8-tetramethylchromane-2-carboxylic acid (Trolox®); Fluorescein Sodium Salt (4nM)
D. Preparation: Pre-heat (37°C) Synergy H1 Microplate reader; Prepare Trolox® standards, sample dilutions, fluorescein solution, and AAPH.
E. Microtitre Plates: Corning 96 Well Black Side/Clear Bottom Microplates

Methods

Solutions of ACB Pisum Sativum Peptide and Trolox® (positive control) were prepared in 75mM potassium phosphate buffer. Materials were prepared at three different concentrations/dilutions. Trolox® was used as a reference for antioxidant capacity and prepared at a concentrations ranging from 12.5µM to 200µM in 75mM potassium phosphate buffer.

For the ORAC assay, 25µL of test material and Trolox® were combined with 150µL of fluorescein in 75mM potassium phosphate buffer and incubated in the Synergy HT Microplate reader at 37°C for 30 minutes. At the end of the incubation period, 25µL of AAPH were pipetted into each well. Fluorescent measurements were then taken every 2 minutes for approximately 2 hours at an excitation wavelength of 485nm and an emissions wavelength of 520nm.

The AUC and Net AUC values of the standards and samples were determined using Gen5 2.0 Data Reduction Software using the below equations:

\[
AUC = 0.5 + \frac{R_2}{R_1} + \frac{R_3}{R_1} + \frac{R_4}{R_1} + \cdots + \frac{R_n}{R_1} \rightarrow \text{Where } R \text{ is fluorescence reading}
\]

\[
\text{Net AUC} = \text{AUC}_\text{sample} - \text{AUC}_\text{blank}
\]

The standard curve was obtained by plotting the Net AUC of different Trolox® concentrations against their concentration. ORAC values of samples were then calculated automatically using the Gen5 software to interpolate the sample’s Net AUC values against the Trolox® standard curve. ORAC measurements for the test material were expressed in micro moles Trolox® equivalents per mL (µMTE), where 1 ORAC unit is equal to 1 µMTE.
Results

**ACB Pisum Sativum Peptide** exhibited very potent antioxidant activity at a 0.3% concentration.

![Antioxidant capacities](image)

**Discussion**

As shown in figure 1, **ACB Pisum Sativum Peptide** exhibited similar strong antioxidant properties similar to 200µM concentration of Trolox, our highest standard used. The antioxidant capacity of **ACB Pisum Sativum Peptide** increased as the concentration increased, as a result we can assure that its ability to minimize oxidative stress is dose dependant.

**ACB Pisum Sativum Peptide** was designed to provide hair conditioning and moisturization and, in addition, act as a film former, however with the present study we can confirm that this unique ingredient is not only capable of providing functional benefits but it is also capable of providing potent antioxidant benefits when added to cosmetic and personal care applications.
**Tradename:** ACB Pisum Sativum Peptide

**Code:** 16810

**Lot #:** 33396

**CAS #:** 90082-41-0

**Test Request Form #:** 949

**Sponsor:** Active Concepts, LLC; 107 Technology Drive Lincolnton, NC 28092

**Study Director:** Erica Segura

**Principle Investigator:** Meghan Darley

**Test Performed:**
Sirius Red/Fast Green Collagen Assay

**Introduction**

Collagen is the main protein of connective tissues, such as skin, bone, tendon and ligament, and the most abundant protein in mammals. Collagen accounts for nearly 25% to 35% of the total human protein content. Collagen is a long, fibrous protein that forms bundles called fibers giving structure and support to cells and tissues. Collagen has great tensile strength and is responsible for skin’s elasticity and, therefore, its degradation leads to wrinkles that accompany aging.

Sirius Red/Fast Green Collagen Assay was conducted to assess the changes in collagen synthesis by **ACB Pisum Sativum Peptide** treated *in vitro* cultured human dermal fibroblasts.

**Assay Principle**

Sirius Red is a unique dye that binds specifically to the helical structure of types I through V collagen, while Fast Green binds to non-collagenous proteins. These two dyes work in conjunction to provide a semi-quantitative method of determining amounts of collagen and non-collagenous proteins in a sample. After staining a sample the dyes are easily extracted and have optical density (OD) absorptions at 540nm (Sirius Red) and 605nm (Fast Green). Protein concentrations are calculated through equations with OD values.
Sirius Red/Fast Green Collagen Analysis

info@activeconceptsllc.com • Phone: +1-704-276-7100 • Fax: +1-704-276-7101

Materials

A. Kit: Sirius Red/Fast Green Collagen Kit (Chondrex; 9046)
B. Incubation Conditions: 37°C at 5% CO₂ and 95% Relative Humidity (RH)
C. Equipment: Forma Humidified Incubator,ESCO Biosafety Laminar Flow Hood, Synergy HT Microplate Reader; Pipettes
D. Cell Line: Normal Human Dermal Fibroblasts (Invitrogen; C-004-5C)
E. Media/Buffers: Complete and Serum-Free Dulbecco’s Modified Eagle Medium (DMEM); Phosphate Buffered Saline (PBS)
F. Culture Plate: Falcon Flat Bottom 24-Well Tissue Culture Treated Plates
G. Reagents: Ascorbic Acid-2-Glucose (AA2G) (100µM); Insulin Growth Factor-1 (IGF-1) (50ng/mL); Glacial Acetic Acid, Ethanol
H. Other: Sterile Disposable Pipette Tips; Wash Bottles

Methods

Human dermal fibroblasts were seeded into 24-well tissue culture plates and allowed to grow to confluency in complete DMEM. 1%, 0.1%, and 0.01% concentrations of ACB Pisum Sativum Peptide were added to the serum-free DMEM and incubated with fibroblasts for 24 hours. AA2G and IGF-1 were used a positive controls.

Media was removed from wells containing adherent fibroblasts and the cells were washed with PBS. 500µl of a cooled 95% ethanol/5% glacial acetic acid solution was added to the wells and incubated for 10 minutes at room temperature. 200µL of the Sirius Red/Fast Green dye solution was added to the fixed cell layer and incubated at room temperature for 30 minutes. The dye solution was removed from the cell layer and washed with water. 1mL of extraction solution was added for color extraction. The optical density was read at 540nm and 605nm on the Synergy HT Microplate Reader.

The protein concentrations of ACB Pisum Sativum Peptide treated-fibroblasts were determined by calculations based on the optical density measurements and expressed in µg.
Results

The data obtained from this study met criteria for a valid assay and the positive and negative controls performed as anticipated.

**ACB Pisum Sativum Peptide** elicited positive effects on collagen synthesis.

Collagen concentration is calculated by the following formula:

\[
\text{Collagen (µg)} = \frac{[OD_{540} - (OD_{605} \times 0.291)]}{0.0378}
\]

\[
\text{Non Collagen Protein (µg)} = \frac{OD_{605}}{0.00204}
\]

**Figure 1:** Collagen and non-collagen protein concentrations
Discussion

As shown in figure 1, **ACB Pisum Sativum Peptide** (code 16810) exhibited positive collagen synthesis activity. The increase in collagen production may lead to improvement in the dermal-epidermal junction integrity as well as an improved scaffolding matrix. For these reasons, we can assume **ACB Pisum Sativum Peptide** (code 16810) is suitable for cosmetic applications designed to boost collagen synthesis to aid in providing a younger and healthier complexion.