### 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 <strong>ACB Pisum Sativum Peptide</strong> 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 <strong>ACB Pisum Sativum Peptide</strong> or 2.0% solution of Wheat Hydrolysate was applied to each strand of test hair. Immediate results showed strands treated with <strong>ACB Pisum Sativum Peptide</strong> 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>Assessment of Hair Characteristics</td>
<td>The results of the assessment indicate that when incorporated into a shampoo, <strong>ACB Pisum Sativum Peptide</strong> is capable of improving hair characteristics 101% more than a control shampoo. When used at 2.0% in a conditioner, <strong>ACB Pisum Sativum Peptide</strong> is capable of improving hair characteristics 61% better than the control conditioner.</td>
</tr>
<tr>
<td>ORAC Assay</td>
<td><strong>ACB Pisum Sativum Peptide</strong> exhibited potent antioxidant activity comparable to 100μM Trolox®. The antioxidant capacity of <strong>ACB Pisum Sativum Peptide</strong> increased as the concentration increased, as a result</td>
</tr>
</tbody>
</table>
we can assure that its ability to minimize oxidative stress is dose dependent.

Sirius Red Fast Green Report Analysis

ACB Pisum 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 Pisum Sativum Peptide is suitable for cosmetic applications designed to boost collagen synthesis to aid in providing a younger and healthier complexion.
ACB Pisum Sativum Peptide Hydrolyzed Protein Hydration Comparison Assay

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 comparable hydrating benefits to the leading hydrolyzed proteins on the market.

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 5.0% Wheat Hydrolysate Solution), and the sample treated with the test material (5.0% ACB Pisum Sativum Peptide). Using a 9003 DPM Nova Impedance Meter, hydration levels of each strand of hair were measured. Both the 5.0% solution of the Wheat Hydrolysate and the 5.0% solution of ACB Pisum Sativum Peptide were shown to increase moisture levels by comparable amount in the hair strand.

Results

![Percent increase in hair hydration using ACB Pisum Sativum Peptide and Wheat Hydrolysate](image)

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 demonstrate that although a novel approach to hydrolysis, when applied to the hair, ACB Pisum Sativum Peptide provides just as much moisture as the Wheat Hydrolysate with proven hydration benefits.
ACB Pisum Sativum Peptide Hair Volumizing Assay
Hydrolyzed Protein Comparison

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

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

Figure 2. Individual strand immediately following treatment with Wheat Hydrolysate (note beading).

Figure 3. Individual strand immediately following treatment with ACB Pisum Sativum Peptide.
ACB Pisum Sativum Peptide Hair Volumizing Assay
Hydrolyzed Protein Comparison

Results (Cont.)

Figure 4. Individual strand four hours after treatment with Wheat Hydrolysate
Figure 5. Individual strand four hours after treatment with ACB Pisum Sativum Peptide

Discussion

Immediate results showed and 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.
Abstract

The condition of the cuticle (the outer most layer of the hair) significantly affects both, manageability and volume of our hair. Overtime as hair becomes damaged the cuticle often lifts as a result of a variety of influences including environment and styling processes. This results in flat, dull hair that is difficult to manage. Improving the body of the hair has been shown to instantly make it appear healthier and more youthful. 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.

A half head study was conducted to determine the comparison of using a shampoo incorporating **ACB Pisum Sativum Peptide** vs. a control shampoo, in addition to the comparison of using a conditioner incorporating **ACB Pisum Sativum Peptide** vs. a control conditioner. The volunteer’s hair was photographed. The images of the half head study were used in conjunction with a sensory study to assess the shine, volume, dry and wet combability, thickness, smoothness, hydration, softness and manageability was also conducted before treatment, after shampoo treatment and blow dry, and again after conditioner treatment and blow dry. Based on the results obtained, **ACB Pisum Sativum Peptide** is capable of enhancing the volume and overall health of the hair perfect for use in anti-aging hair care product lines.

Materials and Methods

The study was conducted using a female participant with medium length hair. The subject was asked to wash her hair and blow dry, as normal, prior to the beginning of the study. Images of the participant’s hair were taken and a sensory evaluation was conducted for baseline measurements.

A half head study was then conducted. After segmenting the hair in half, one half of the participant’s hair was shampooed with 2.0% **ACB Pisum Sativum Peptide** in Biolâge Shampoo and the other half of the hair with the untreated Biolâge control shampoo. Following the shampoo, the participant was asked to comb out wet hair for a sensory assessment of wet combability, then the participant’s hair was blown dry using a flat paddle brush on both sides of the head. Once the hair was completely dry, the participant was asked to assess the volume, shine, dry combability, thickness, smoothness, hydration, softness and manageability of both halves of her hair. Assessments were made using a rubric from 1 to 10, with 1 being the lowest and 10 being the highest.

Following the assessment of the shampoos, a half head study to evaluate two conditioners was conducted. After segmenting the hair in half, one half of the participant’s hair was conditioned with 2.0% **ACB Pisum Sativum Peptide** in Biolâge Conditioner and the other half of the hair with the untreated Biolâge control conditioner. Following the conditioning of the participant’s hair, she was asked to comb out the wet hair for a sensory assessment of wet combability, then the participant’s hair was blown dry using a flat paddle brush on both sides of the head. Once the hair was completely dry, the participant was asked to assess the volume, shine, dry combability, thickness,
smoothness, hydration, softness and manageability of both halves of her 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></th>
<th>Baseline Assessment of Untreated Hair</th>
<th>Assessment of Half Head Shampooed with Untreated Control</th>
<th>Assessment of Half Head Shampooed with Pisum Sativum Peptide</th>
<th>Assessment of Half Head Conditioned with Untreated Control</th>
<th>Assessment of Half Head Conditioned with Pisum Sativum Peptide</th>
</tr>
</thead>
<tbody>
<tr>
<td>Shine</td>
<td>5</td>
<td>4</td>
<td>7</td>
<td>7</td>
<td>10</td>
</tr>
<tr>
<td>Volume</td>
<td>4</td>
<td>4</td>
<td>7</td>
<td>3</td>
<td>8</td>
</tr>
<tr>
<td>Dry Combability</td>
<td>2</td>
<td>2</td>
<td>6</td>
<td>6</td>
<td>10</td>
</tr>
<tr>
<td>Wet Combability</td>
<td>1</td>
<td>2</td>
<td>5</td>
<td>6</td>
<td>10</td>
</tr>
<tr>
<td>Thickness</td>
<td>6</td>
<td>8</td>
<td>7</td>
<td>6</td>
<td>7</td>
</tr>
<tr>
<td>Smoothness</td>
<td>5</td>
<td>5</td>
<td>9</td>
<td>8</td>
<td>10</td>
</tr>
<tr>
<td>Hydration</td>
<td>7</td>
<td>6</td>
<td>10</td>
<td>7</td>
<td>10</td>
</tr>
<tr>
<td>Softness</td>
<td>7</td>
<td>3</td>
<td>10</td>
<td>6</td>
<td>10</td>
</tr>
<tr>
<td>Managability</td>
<td>3</td>
<td>4</td>
<td>7</td>
<td>6</td>
<td>9</td>
</tr>
<tr>
<td>Mean</td>
<td>4</td>
<td>4</td>
<td>8</td>
<td>6</td>
<td>9</td>
</tr>
</tbody>
</table>

Table 1. Results of Sensory Assessment of Hair

---

**Chart 1.** Rating of hair characteristics following sensory assessment
ACB Pisum Sativum Peptide
Assessment of Hair Characteristics

Figure 1. Full Head Baseline Photo of Untreated Hair

Figure 2. Half-Head Photo of Hair Treated with Test and Control Shampoos

Half-Head Treated with 2.0%
ACB Pisum Sativum Peptide
in Biolage Shampoo

Half-Head Treated with
Untreated Biolage
Control Shampoo

Information contained in this technical literature is believed to be accurate and is offered in good faith for the benefit of the customer. The company, however, cannot assume any liability or risk involved in the use of its chemical products since the conditions of use are beyond our control. Statements concerning the possible use of our products are not intended as recommendations to use our products in the infringement of any patent. We make no warranty of any kind, expressed or implied, other than that the material conforms to the applicable standard specification.
ACB Pisum Sativum Peptide
Assessment of Hair Characteristics

Figure 3. Half-Head Photo of Hair Treated with Test and Control Conditioners

Percent (%) Difference of Sensory Hair Assessment

Chart 2. Hair Assessment results for sensory characteristics
**Discussion**

The results of the assessment indicate that when incorporated into a shampoo, **ACB Pisum Sativum Peptide** is capable of improving hair characteristics 44% more effectively than a control shampoo. When used in a shampoo **ACB Pisum Sativum Peptide** improved shine, volume, softness and dry and wet combability 43%, 43%, 70%, 67% and 60% respectively, better than the control shampoo. Furthermore, Hydration and manageability were considered about 40% better when using the shampoo containing the test material. These results can be further supported by figure 2, where clearly the half of the subject's head treated with 2.0% **ACB Pisum Sativum Peptide** appears more voluminous, shiny and soft.

When used at 2.0% in a conditioner, **ACB Pisum Sativum Peptide** is capable of improving hair characteristics 36% better than the control conditioner. Dry and Wet combability and softness were all improved by 40% when using the conditioner with **ACB Pisum Sativum Peptide** in comparison to the control conditioner. Most impressively, the treated conditioner improved volume 75% more effectively than the untreated conditioner. Shine, smoothness, hydration, softness and manageability of the half head using the treated conditioner were all ranked about 30% higher on average than the untreated side of the head. These results can be further supported by figure 3, where clearly the half of the subject's head treated with 2.0% **ACB Pisum Sativum Peptide** appears more voluminous, manageable and soft.

For this reason, **ACB Pisum Sativum Peptide** would be an ideal product to use in cosmetic applications designed for anti-aging hair care that yields voluminous, soft and shiny hair aesthetics.
**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 Pismum 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:

\[ AUC = 0.5 + \frac{R2}{R1} + \frac{R3}{R1} + \frac{R4}{R1} + \cdots + \frac{Rn}{R1} \rightarrow \text{Where } R \text{ is fluorescence reading} \]

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

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.

Information contained in this technical literature is believed to be accurate and is offered in good faith for the benefit of the customer. The company, however, cannot assume any liability or risk involved in the use of its chemical products since the conditions of use are beyond our control. Statements concerning the possible use of our products are not intended as recommendations to use our products in the infringement of any patent. We make no warranty of any kind, expressed or implied, other than that the material conforms to the applicable standard specification.
Results

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

![Antioxidant capacities](image)

**Figure 1**: Antioxidant capacities

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.
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:

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

\[
Non \ Collagen \ Protein \ (\mu g) = \frac{OD_{605}}{0.00204}
\]

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

As shown in figure 1, **ACB Pism 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 Pism Sativum Peptide** (code 16810) is suitable for cosmetic applications designed to boost collagen synthesis to aid in providing a younger and healthier complexion.