**ACB Quinoa Protein**

**Efficacy Data**

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<tr>
<th>Name of Study</th>
<th>Results</th>
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<td><strong>Cellular Viability Assay</strong></td>
<td>As shown in figure 1, ACB Quinoa Protein exhibited comparable results by increasing cell metabolism. An increase in fluorescent signal indicates an increase in cellular metabolism and viability. ACB Quinoa Protein does not appear to have negative effects on cellular metabolism and can safely be used in cosmetic materials.</td>
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<td><strong>Hydration Assay</strong></td>
<td>Both the innovative hydrolysis method used in the production of ACB Quinoa Protein 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 Quinoa Protein provides just as much moisture as the Wheat Hydrolysate with proven hydration benefits.</td>
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<td><strong>TEWL</strong></td>
<td>As shown in Figure 1, results indicate continuous improvements in the barrier of the skin throughout the 3 week test period. After one week, the solution containing 2.0% ACB Quinoa Protein decreased TEWL 15% more effectively than the base lotion alone. After three weeks, the solution containing 2.0% ACB Quinoa Protein demonstrated even more effective barrier protection, decreasing TEWL 21% better than the base lotion alone. When compared to the untreated control, the solution containing 2.0% ACB Quinoa Protein decreased transepidermal water loss by 26% after one week and by 22% after three weeks.</td>
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Cellular Viability Assay Analysis

Tradename: ACB Quinoa Protein

Code: 20037

CAS #: 100209-45-8

Test Request Form #: 1270

Lot Number: NC-150122-C

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

Study Director: Erica Segura

Principal Investigator: Meghan Darley

Test Performed:
Cellular Viability Assay

Introduction

The cellular viability assay is useful for quantitatively measuring cell-mediated cytotoxicity, cell proliferation and mitochondrial metabolic activity. Increased metabolism in a cell indicates ample cellular respiration and adenosine triphosphate (ATP) production. ATP is the molecular energy of cells and is required in basic cell function and signal transduction. A decrease is ATP levels indicates cytotoxicity and decreased cell function while an increase in ATP levels indicates healthy cells.

The cellular viability assay was conducted to assess the ability of ACB Quinoa Protein to increase cellular metabolic activity in cultured dermal fibroblasts.

Assay Principle

The assay utilizes a nonfluorescent dye, resazurin, which is converted to a fluorescent dye, resorufin, in response to chemical reduction of growth medium from cell growth and by respiring mitochondria. Healthy cells that are in a proliferative state will be able to easily convert resazurin into resorufin without harming the cells. This method is a more sensitive assay than other commonly used mitochondrial reductase dyes such as MTT. An increase in the signal generated by resazurin-conversion is indicative of a proliferative cellular state.
Materials

A. Kit: Cellular Senescence Assay Kit (Chemicon® International; KAA002)
B. Incubation Conditions: 37°C at 5% CO₂ and 95% relative humidity (RH)
C. Equipment: Forma humidified incubator; ESCO biosafety laminar flow hood; Light microscope; Pipettes
D. Cell Line: Normal Human Dermal Fibroblasts (NHDF) (Lonza; CC-2511)
E. Media/Buffers: Dulbecco’s Modified Eagle Medium (DMEM); Penicillin-Streptomycin (50U-50mg/mL); Fetal Bovine Serum (FBS); Phosphate Buffered Saline (PBS)
F. Culture Plate: Falcon flat bottom 6-well tissue culture treated plates
G. Reagents: 5-bromo-4-chloro-indolyl-β-D-galactopyranoside (X-gal)
H. Other: Sterile disposable pipette tips; wash bottles; 15mL conical tubes, 1.5mL microcentrifuge tubes

Methods

Human dermal fibroblasts were seeded into 96-well tissue culture plates and allowed to grow to confluency in complete DMEM. A 10-fold serial dilution was performed resulting in ACB Quinoa Protein concentrations on 1%, 0.1%, and 0.01% in complete DMEM and incubated with fibroblasts for 24 hours.

Ten microliters of viability reagent was added to 90µL of cell culture media in culture wells.
Results

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

**ACB Quinoa Protein** exhibited significant effects on cellular metabolism compared to the control.

Cellular metabolism results are expressed as a percentage of the control.

Figure 1: Cellular Metabolism of **ACB Quinoa Protein**-treated fibroblasts expressed in terms of percent of control.

Discussion

As shown in figure 1, **ACB Quinoa Protein** exhibited comparable results by increasing cell metabolism. An increase in fluorescent signal indicates an increase in cellular metabolism and viability. **ACB Quinoa Protein** does not appear to have negative effects on cellular metabolism and can safely be used in cosmetic materials.
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, ACB Quinoa Protein. 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.

Abstract

The hair samples used in this study were tested using identical intervals and percentages of two protein hydrolysates, ACB Quinoa Protein 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 (2.0% Wheat Hydrolysate in an Aqueous Solution), and the sample treated with the test material (2.0% ACB Quinoa Protein in an Aqueous Solution). Using a 9003 DPM Nova Impedance Meter, hydration levels of each strand of hair were measured. Both the 2.0% Wheat Hydrolysate Aqueous Solution and the 2.0% ACB Quinoa Protein Aqueous Solution were shown to increase moisture levels by comparable amounts in the respective hair strands.

Results

% Increase in Hair Hydration

<table>
<thead>
<tr>
<th>Untreated Control</th>
<th>2.0% Wheat Hydrolysate</th>
<th>2.0% ACB Quinoa Protein</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>12</td>
<td>14</td>
</tr>
<tr>
<td>2</td>
<td>10</td>
<td>12</td>
</tr>
<tr>
<td>4</td>
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</tr>
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<td>12</td>
<td>0</td>
<td>2</td>
</tr>
</tbody>
</table>

Figure 1. Percent increase in hair hydration using ACB Quinoa Protein and Wheat Hydrolysate

Discussion

Both the innovative hydrolysis method used in the production of ACB Quinoa Protein and the traditional method of hydrolysis induced when creating Wheat Hydrolysate yeild 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 Quinoa Protein provides just as much moisture as the Wheat Hydrolysate with proven hydration benefits.
Transepidermal Water Loss Assay

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Results

**Figure 1:** Improvements in barrier function following application of the test materials after a period of 3 weeks.

**Discussion**

As shown in Figure 1, results indicate continuous improvements in the barrier of the skin throughout the 3 week test period. After one week, the solution containing 2.0% **ACB Quinoa Protein** decreased TEWL 15% more effectively than the base lotion alone. After three weeks, the solution containing 2.0% **ACB Quinoa Protein** demonstrated even more effective barrier protection, decreasing TEWL 21% better than the base lotion alone.

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