Active Concepts
Tomorrow's Vision... *Today!*®

ACB Quinoa Protein Efficacy Data

Code: 20037
INCI Name: Hydrolyzed Quinoa
CAS #: 100209-45-8
EINECS #: 309-353-8

Type of Study	Results
Cellular Viability Assay	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.
Hydration Assay	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.
TEWL	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.

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

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info@activeconceptsllc.com • +1 (704)-276-7100 • Fax: +1 (704)-276-71

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*
Principle 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.

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Materials

A.	**Kit:**	Cellular Senescence Assay Kit (Chemicon® International; KAA002)
B.	**Incubation Conditions:**	37°C at 5% CO_2 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.

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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.

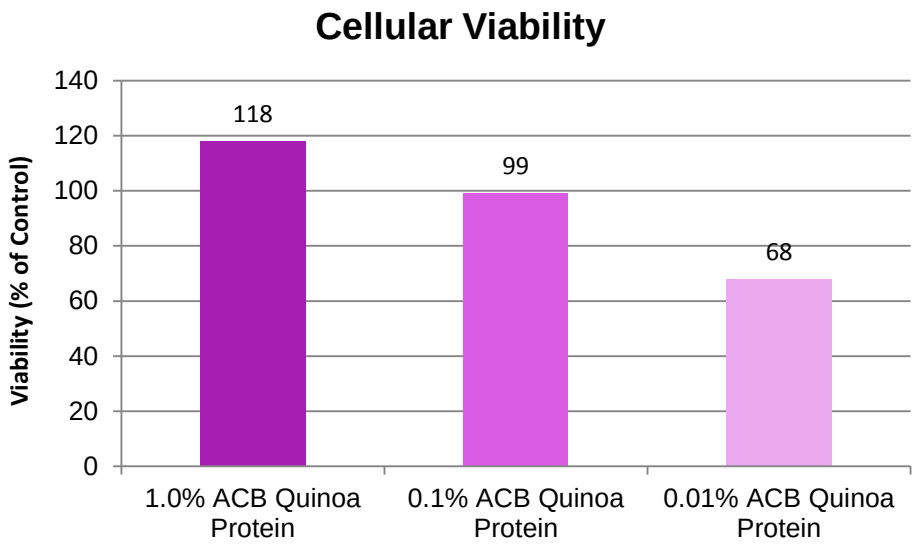

Figure 1: Cellular Metabolism of **ACB Quinoa Protein**-treated fibroblasts expressed in terms of percent of control.

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.

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ACB Quinoa Protein Hydration Comparison Assay

Code: 20037
INCI Name: Hydrolyzed Quinoa
Suggested Use Levels: 1.0 - 10.0%
TRF#: S7

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, hyrolysis was induced using acid, water, or fermentation. Active Concepts has impelemented 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.

Materials and Methods

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

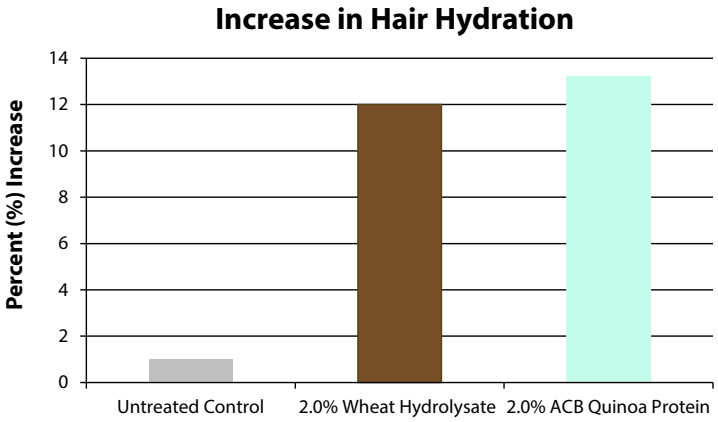

Figure 1.Percent increase in hair hydration using **ACB Quinoa Protein** and Wheat Hydrolysate

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.

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Transepidermal Water Loss Study

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Tradename: ACB Quinoa Protein

Code: 20037

CAS #: 100209-45-8

Test Request Form #: 1271

Lot Number: NC-150122-C

Sponsor: *Active Concepts, LLC; 107 Technology Drive Lincolnton, NC 28092*
Study Director: *Erica Segura*
Principle Investigator: *Meghan Darley*

Test Performed: Transepidermal Water Loss Study

Introduction

An *in-vivo* study was conducted over a period of three weeks to evaluate the ability of **ACB Quinoa Protein** to enhance barrier function through reduction in Transepidermal Water Loss (TEWL). Results indicate that this material is capable of efficiently reducing TEWL which allows moisture retention.

Materials

A. Equipment: DermaLab Skin Combo

Methods

Ten volunteers M/F between the ages of 23 and 45 and who were known to be free of any skin pathologies participated in this study. A Dermalab Combo was used to measure TEWL on the subject's volar forearms. The instrument consists of a probe that is based upon the vapor gradient with an open chamber. This open chamber design maintains the free natural evaporation from the skin without interfering with the environment over the measurement area. This ensures unbiased and accurate readings. Operation of the water loss module is fully menu drive, allowing for pre-setting and standard deviation or measurement time. Baseline TEWL readings were taken on day one of the study.

Following initial measurements, all subjects were asked to apply 5milligrams of each test material on their volar forearms. Measurements were taken immediately after application of the test materials and then weekly for three weeks. The test material consisted of 2% **ACB Quinoa Protein** in a base lotion.

For added perspective, measurements of an untreated test site and a site treated with a base lotion (Cetaphil Moisturizing for All Skin Types) were recorded.

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Results

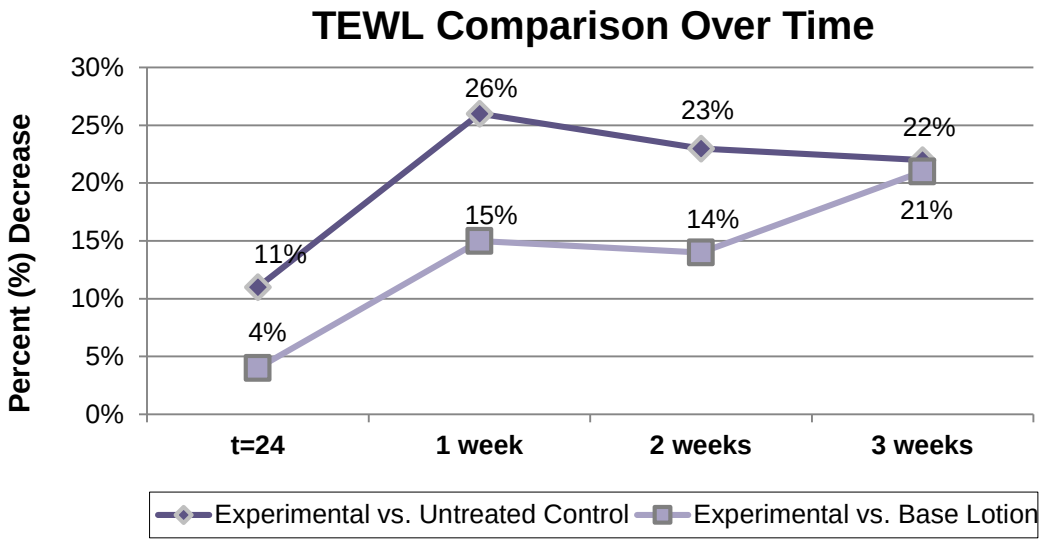

Figure 1: Improvements in barrier function following application of the test materials after a period of 3 weeks.

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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