ACB Tonka Bean Bioferment PF Efficacy Data

Code: 20431PF
INCI Name: Lactobacillus/Dipteryx Odorata Seed Ferment Filtrate
CAS #: 90028-06-1
EINECS #: 289-793-4

Name of Study	Results
Fluorometer Study	The results indicate that **ACB Tonka Bean Bioferment PF** is capable of emitting light in the visible spectrum. The continuous lines on the graphs indicate that the emission of light begins at 405 nm, and continues on through 590 nanometers. This means that light was also emitted at 420 nm, which is blue light. When tested in water as the solvent, 10% **ACB Tonka Bean Bioferment PF** emitted so much light in the visible spectra that it was actually too high to detect using the fluorometer.
Moisturization Assay	**ACB Tonka Bean Bioferment PF** was designed to provide moisturizating benefits, however with the present study we can confirm that this succulent botanical ingredient is not only capable of providing protective benefits but also ideal for moisturizing and skin hydrating personal care applications.
ORAC Assay	As shown in figure 1, **ACB Tonka Bean Bioferment PF** exhibited antioxidant activity comparable to 200µM Trolox®. The antioxidant capacity of **ACB Tonka Bean Bioferment PF** increased as the concentration increased, as a result we can assure that its ability to minimize oxidative stress is dose dependent. **ACB Tonka Bean Bioferment PF** was designed to have anti-wrinkle and chromatherapy properties. 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 applications.

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Fluorometer Study

Tradename: ACB Tonka Bean Bioferment PF

Code: 20431PF

CAS #: 90028-06-1

Test Request Form #: 2459

Lot #: 23736P

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

Test Performed:
Flurometer

Introduction

Diffusers are intended to optimize the appearance of our skin by masking dullness and minimizing the appearance of fine lines that are only visible by the shadows that form on the skin when light is reflected. However they merely diffuse light and cannot fully counteract dullness. Instead of using diffusers, **ACB Tonka Bean Bioferment PF** utilizes photo-activation technology to emit light in the visible spectra. It contains fermented phyto-compounds from tonka beans that are capable of absorbing invisible light in the UV spectra and then converting it to visible light. This study was performed to illustrate that following excitation, **ACB Tonka Bean Bioferment PF** is capable of emitting light in the visible spectra.

Methods

A fluorometer (Fluoroscan Ascent FL, Labsystems) was used to determine the colored light emission properties of ACB Tonka Bean Bioferment PF. Concentrations of 0.1%, 1.0% and 10% **ACB Tonka Bean Bioferment PF** were prepared in solutions where either water or an equal mixture of water and ethanol was used as the solvent. The fluorescent properties of the solvents were also determined as a control. Samples were excited at a wavelength of 320 nm (the maximum absorbance for this material). Emission wavelengths were then tested at 405 nm (violet), 518 nm (green), 590 nm (yellow) and 646 nm (red).

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Results

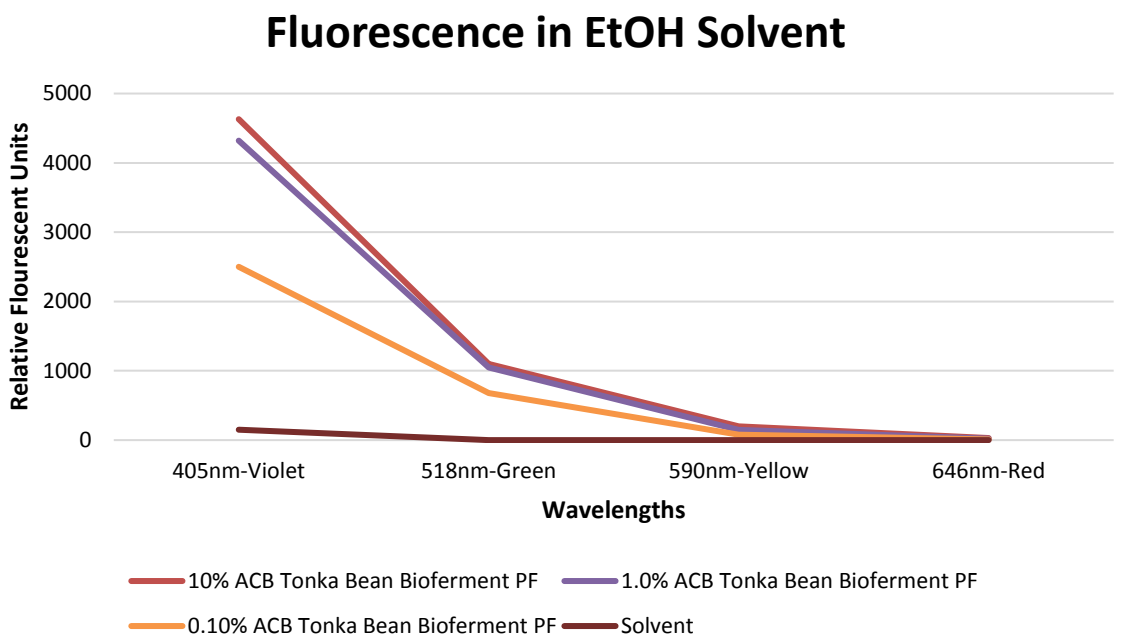

Figure 1: Fluorometer Results for samples of **ACB Tonka Bean Bioferment PF** in ethanol water mixture.

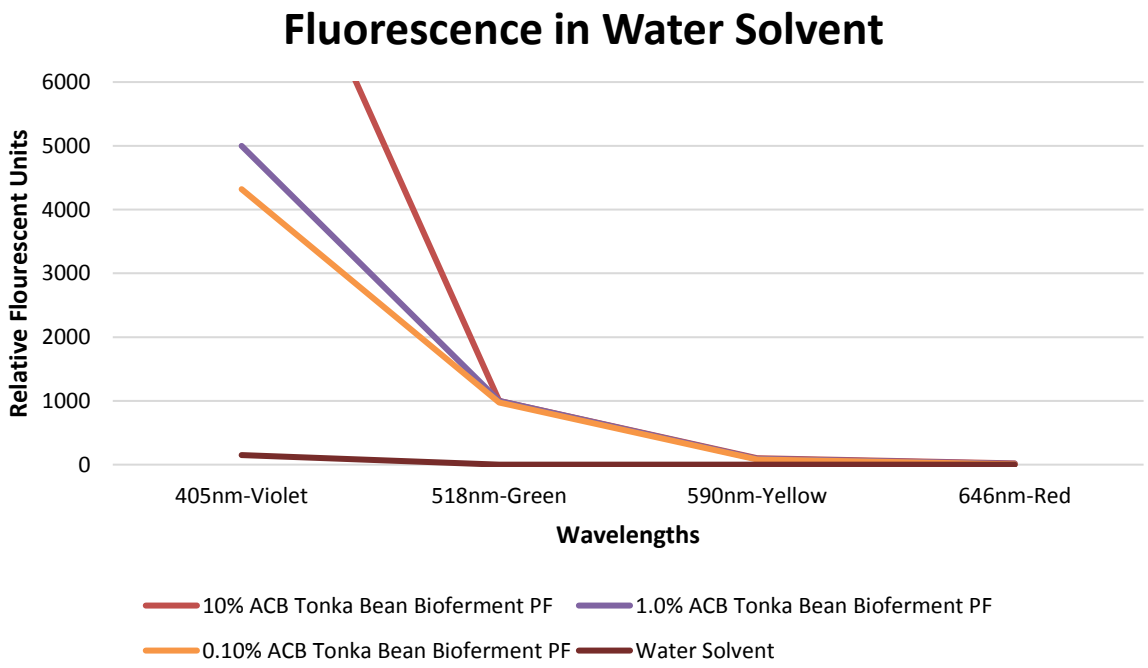

Figure 2: Fluorometer Results for samples of **ACB Tonka Bean Bioferment PF** in water solvent.

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Discussion

The results indicate that **ACB Tonka Bean Bioferment PF** is capable of emitting light in the visible spectrum. The continuous lines on the graphs indicate that the emission of light begins at 405 nm, and continues on through 590 nanometers. This means that light was also emitted at 420 nm, which is blue light. When tested in water as the solvent, 10% **ACB Tonka Bean Bioferment PF** emitted so much light in the visible spectra that it was actually too high to detect using the fluorometer.

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Moisturization/ Hydration Assay

Tradename: ACB Tonka Bean Bioferment PF

Code: 20431PF

CAS #: 90028-06-1

Test Request Form #: 935

Lot #: 37408

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

Test Performed:
Moisturization/ Hydration Assay

Introduction

An *in-vivo* study was conducted over a period of three weeks to evaluate the moisturization benefits of **ACB Tonka Bean Bioferment PF**. 10 M/F subjects between the ages of 23-45 participated in the study. Results indicate that this material is capable of significantly increasing moisturization compared to the control.

The Moisturizaiton Assay was conducted to assess the moisturizing ability of **ACB Tonka Bean Bioferment PF**.

Materials

A. Equipment: DermaLab Skin Combo (Hydration/ Moisture Pin Probe)

Methods

The moisture module provides information about the skin's hydration by measuring the conducting properties of the upper skin layers when subjected to an alternating voltage. The method is referred to as a conductance measurement and the output is presented in the unit of uSiemens (uS). A moisture pin probe is the tool used to gather hydration values.

10 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 Corneometer was used to measure the moisture levels on the subject's volar forearms. The Corneometer is an instrument that measures the amount of water within the skin. The presence of moisture in the skin improves conductance therefore results in higher readings than dry skin. Therefore the higher the levels of moisture, the higher the readings from the Corneometer will be. Baseline moisturization readings were taken on day one of the study.

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Following initial measurements, all subjects were asked to apply 2 mg of each test material on their volar forearms. Measurements were taken immediately after application of test materials and then weekly for 3 weeks. The test material consisted of 2.0% **ACB Tonka Bean Bioferment PF** 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.

Results

ACB Tonka Bean Bioferment PF showed very high moisturizing capabilities at a 2.0% concentration. Please note, each value is an average of three consecutive readings per test site.

Moisturization		T = 0	T = 24 Hours	T = 1 Week	T = 2 Weeks	T = 3 Weeks	T = 4 Weeks	T = -24 Hours	T = -1 Week	T = -2 Weeks
Panelist 1	Experimental	119	125	149	199	211	215	200	88	83
	Base Lotion	109	125	132	139	192	201	113	89	86
	Untreated	108	97	102	111	115	130	128	105	94
Panelist 2	Experimental	123	187	211	250	260	270	115	101	98
	Base Lotion	162	150	157	185	195	215	149	125	125
	Untreated	121	107	87	88	111	220	103	95	94
Panelist 3	Experimental	107	116	120	215	220	235	175	165	160
	Base Lotion	72	100	132	142	155	185	151	107	100
	Untreated	126	139	117	132	130	165	232	162	154
Panelist 4	Experimental	97	112	142	202	215	225	153	132	101
	Base Lotion	83	115	122	128	142	155	152	151	74
	Untreated	62	78	74	92	89	96	88	80	151
Panelist 5	Experimental	99	135	156	200	250	265	157	149	147
	Base Lotion	87	107	175	181	187	195	103	102	74
	Untreated	89	92	102	112	109	115	103	83	83
Panelist 6	Experimental	68	125	155	167	177	182	138	120	118
	Base Lotion	69	115	127	135	143	152	105	110	99
	Untreated	70	59	74	87	110	82	80	69	87
Panelist 7	Experimental	145	164	170	200	229	230	225	125	118
	Base Lotion	98	105	109	115	125	168	144	106	104
	Untreated	139	124	121	132	145	126	125	157	132
Panelist 8	Experimental	140	182	220	235	240	247	238	196	188
	Base Lotion	148	151	154	200	207	226	255	204	181
	Untreated	106	103	106	158	184	107	106	109	106
Panelist 9	Experimental	79	146	178	197	199	200	105	92	91
	Base Lotion	62	112	74	176	179	180	80	75	59
	Untreated	82	85	91	123	125	177	98	94	92
Panelist 10	Experimental	97	110	132	165	185	192	111	90	84
	Base Lotion	102	111	121	185	192	203	127	100	89
	Untreated	93	92	99	107	110	101	134	105	102
Number of Panelists		10	10	10	10	10	10	10	10	10

Chart 1. Panelist Moisturization Measurements

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Averages	T = 24 Hours	T = 1 Week	T = 2 Weeks	T = 3 Weeks	T = 4 Weeks	T = -24 Hours	T = -1 Week
2.0% ACB Tonka Bean Bioferment PF in Base Lotion	140.2	163.3	203	218.6	226.1	161.7	125.8
Base Lotion	119.1	130.6	158.6	171.7	188	137.9	116.9
Untreated	97.6	97.3	114.2	122.8	131.9	119.7	105.9

Chart 2. Average Moisture Increase and Regression Scores of Individual Test Sites

Percent (%) Change	T = 24 Hours	T = 1 Week	T = 2 Weeks	T = 3 Weeks	T = 4 Weeks	T = -24 Hours	T = -1 Week
Base Lotion vs. Untreated	22.02	33.91	38.87	39.82	42.53	15.20	10.38
2.0% ACB Tonka Bean Bioferment PF + Base Lotion vs. Untreated	43.64	67.83	77.75	78.01	71.41	35.08	18.79
2.0% ACB Tonka Bean Bioferment PF in Base Lotion vs. Base Lotion	17.71	25.32	27.99	27.31	20.26	17.25	7.613

Chart 3. Comparative Moisture Increase and Regression Scores Between Individual Test Sites

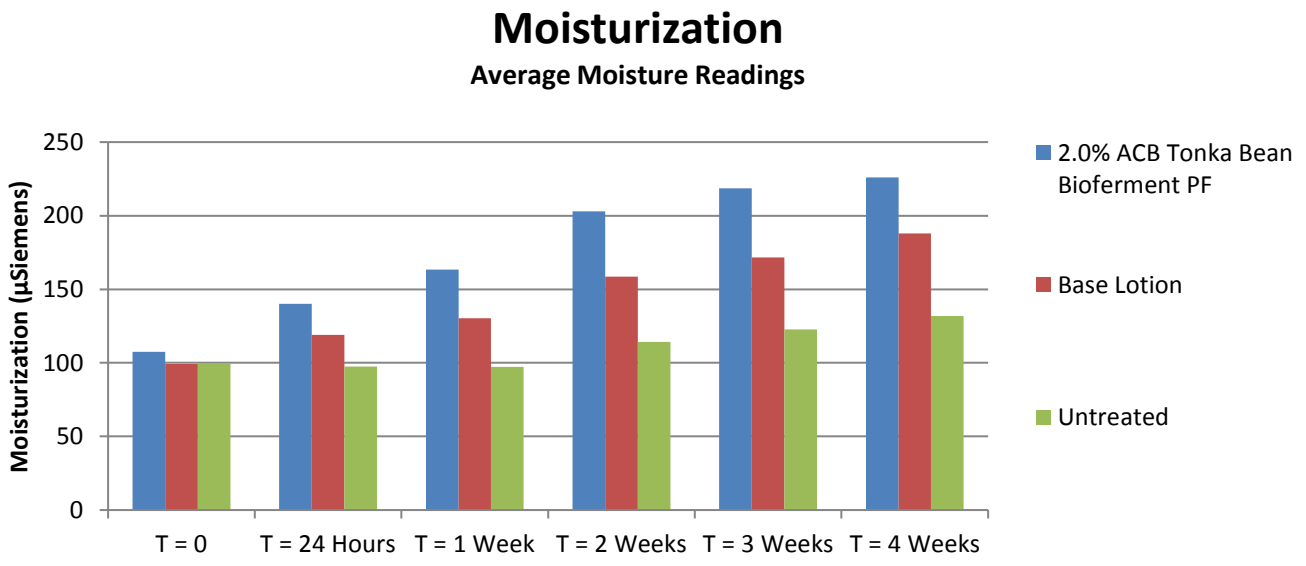

Figure 1. Average increase in moisturization per test site

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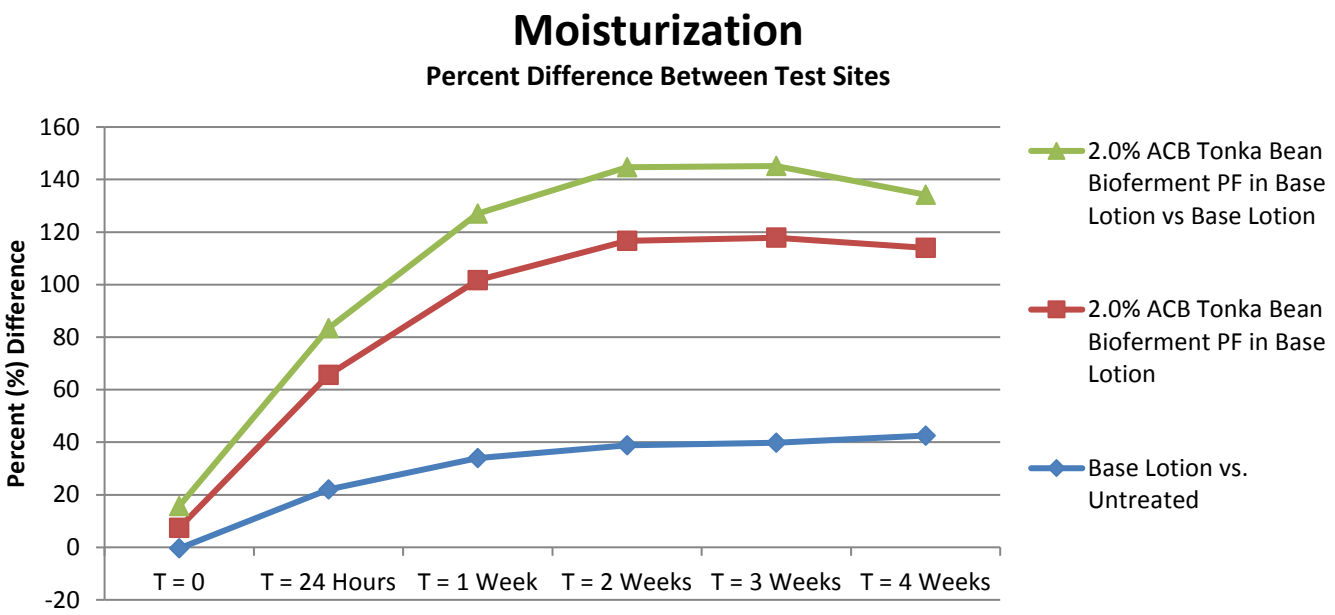

Figure 2. Percent difference in moisturization between two test sites over four weeks

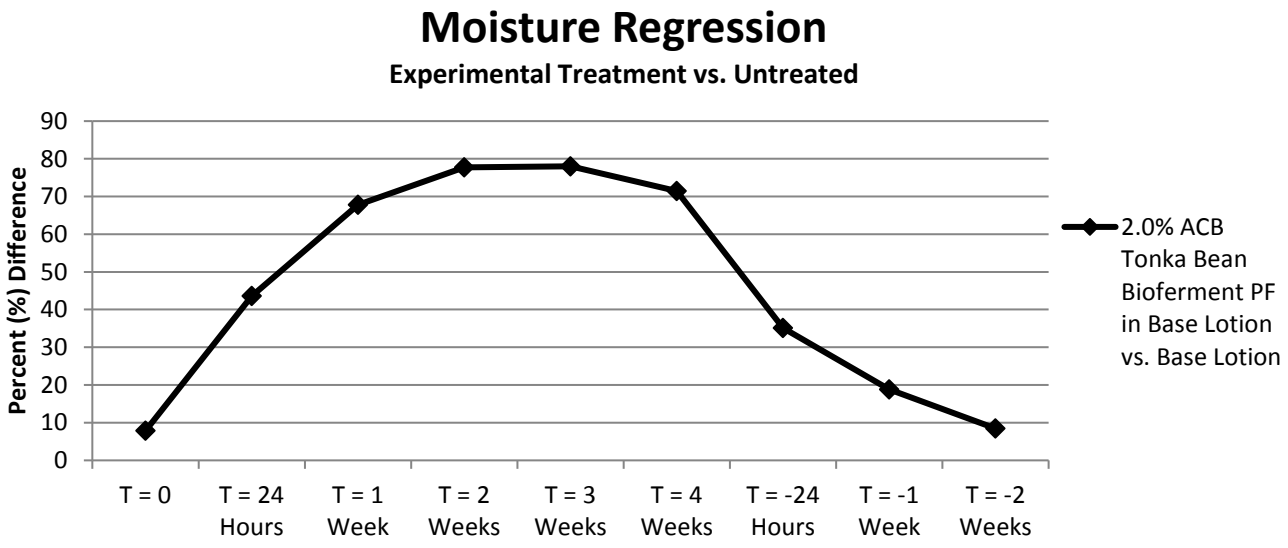

Figure 3. Regression in skin moisturization after application of experimental material ceased

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Discussion

As evidenced in a 4 week efficacy study of **ACB Tonka Bean Bioferment PF** on skin, moisture levels were improved by 43.6% after 24 hours and by 71.41% after 4 weeks when compared to the untreated control. Comparisons of the base lotion to the Experimental Lotion containing 2.0% **ACB Tonka Bean Bioferment PF** demonstrate the experimental material moisturized the skin 21.0% better after 24 hours. After four weeks the base lotion containing 2.0% **ACB Tonka Bean Bioferment PF** moisturized skin 20.2% better than the base lotion alone. Results indicate that **ACB Tonka Bean Bioferment PF** is capable of increasing moisturization when compared to both the untreated control as well as the base lotion.

Furthermore, when examining the moisture levels on the skin after application of test materials stopped, it was determined that **ACB Tonka Bean Bioferment PF** is capable of sustaining increased skin moisturization when compared to the skin site that remained untreated through the duration of the study. After 24 hours, the site testing 2.0% **ACB Tonka Bean Bioferment PF + Base Lotion** was approximately 35.0% more moisturized than the site which did not receive treatment. After one week, the experimental test site was still yielding moisturization results that were 18.7% higher than the untreated site. Additionally, in comparison to the site tested with the base lotion alone, the site treated with 2.0% **ACB Tonka Bean Bioferment PF + Base Lotion** moisturized the skin 17.2% better after 24 hours and was still 7.61% more effective in moisturizing the skin when reading were taken one week after the applications of both test materials ceased.

ACB Tonka Bean Bioferment PF was designed to provide moisturizating benefits, however with the present study we can confirm that this succulent botanical ingredient is not only capable of providing protective benefits but also ideal for moisturizing and skin hydrating personal care applications.

Oxygen Radical Absorbance Capacity (ORAC) Assay

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Tradename: ACB Tonka Bean Bioferment PF

Code: 20431PF

CAS #: 90028-06-1

Test Request Form #: 232

Lot #: 25387

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

Test Performed:
Oxygen Radical Absorbance Capacity (ORAC)

Introduction

Reactive oxygen species (ROS) are generated by normal cellular processes, environmental stresses, and UV irradiation. ROS are detrimental 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 Tonka Bean Bioferment PF**.

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.

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Materials

A. Equipment:	Synergy H1 Microplate reader (BioTek Instuments, Winooski, VT); Gen5 software (BioTek Instuments, Winooski, VT); Pipettes
B. Buffers:	75mM Potassium Phosphate (pH 7.4); Deionized H_2O
C. Reagents:	2,2′-Azobis(2-methylpropionamidine) 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 Tonka Bean Bioferment PF** 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:

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

$$Net\ AUC = AUC_{sample} - AUC_{blank}$$

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 (µMTE), where 1 ORAC unit is equal to 1 µMTE.

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Results

ACB Tonka Bean Bioferment PF began exhibiting antioxidant activity at a 0.0125% concentration.

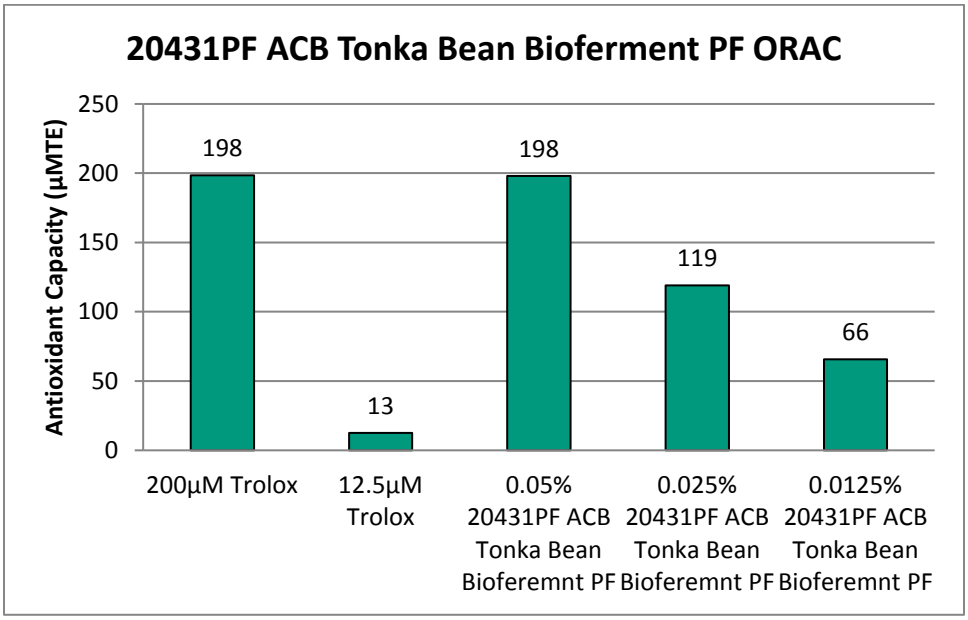

Figure 1: Antioxidant capacities

Discussion

As shown in figure 1, **ACB Tonka Bean Bioferment PF** exhibited antioxidant activity comparable to 200µM Trolox®. The antioxidant capacity of **ACB Tonka Bean Bioferment PF** increased as the concentration increased, as a result we can assure that its ability to minimize oxidative stress is dose dependent.

ACB Tonka Bean Bioferment PF was designed to have anti-wrinkle and chromatherapy properties. 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 applications.