

**Title**

Effect of Jojoba Esters on Skin Barrier Function, Skin Hydration, and Consumer Preference

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Abstract

Previous literature shows that jojoba oil and jojoba oil derivatives have multiple skin benefits associated with anti-inflammation, barrier function, and skin hydration. The current research explores the action of Jojoba Esters with different melting points on the improvement of barrier function after sodium lauryl sulfate (SLS) insult, reduction of barrier disruption when applied prior to SLS insult, and on skin hydration as compared to known petrolatum-alternatives. In addition, a consumer preference study was carried out comparing Jojoba Esters with a known marketed petrolatum alternative ingredient. The data show that Jojoba Esters have the capability of reducing damage to the skin barrier, as measured by TEWL, in a dose dependent manner, and can accelerate the recovery of the SLS damaged skin barrier. The data also show that the Jojoba Esters, when added to a vehicle formula, produced statistically significant increases in skin hydration when compared to the vehicle and to the marketed petrolatum-alternative ingredients formulated in the same vehicle. Moreover, the formula containing the Jojoba Esters produced levels of skin hydration that were not statistically significantly different from 10% petrolatum formulated into the same vehicle. Additionally, the consumer preference study showed that the subjects preferred the Jojoba Esters formula over the petrolatum alternative ingredient formula with regard to skin moistness, suppleness, and smoothness/softness as well as stickiness and residue of the formulas immediately after application.

Introduction

There is a substantial body of evidence supporting the characterization of atopic dermatitis by epidermal barrier dysfunction,¹⁻³ leading to an increase in transepidermal water loss (TEWL).⁴⁻⁶ In atopic dermatitis, a role for stratum corneum lipids in the etiology of the disease has evolved, with special interest in the ceramides.⁷⁻¹⁰ This strong relationship with ceramides and atopic dermatitis has led to the inclusion of ceramides in topical formulations for the treatment of this skin condition.¹¹

In addition to ceramides, a number of ingredients over the years have been formulated into topical products to help restore skin barrier integrity. These have included petrolatum, silicones, glycerin, mineral oil and lipids.¹² Another class of ingredients with properties similar to petrolatum, but made from botanical sources, are the Jojoba Esters. These are a family of unique wax esters, with different melting points and degrees of firmness. Jojoba Esters are derived from the interesterification of jojoba oil with fully hydrogenated jojoba oil,¹³ yielding a mixture of wax ester, C36 –C46, which are “*trans*” free esters in nature.¹⁴ Jojoba Esters have been used in a variety of personal care product categories for their ability to provide emolliency and oxidative stability,¹⁴⁻¹⁵ including but not limited to, moisturizers, hair care, and color cosmetics¹⁵ such as lipsticks.¹⁶ In the present paper, we demonstrate the ability of Jojoba Esters to increase skin barrier recovery and reduce skin barrier disruption due to sodium lauryl sulfate (SLS), increase skin hydration similar to petrolatum, and enhance consumer preference.

Jojoba Esters with three different melting points were studied in five separate vehicle-controlled *in vivo* studies: (1) an initial barrier function study in which the skin was treated with Jojoba Esters before SLS exposure followed by (2) a dose response study to determine the minimum concentration of Jojoba Esters to produce the maximum barrier protection from SLS,

(3) a barrier recovery study in which the skin was treated with Jojoba Esters following SLS exposure, (4) a skin hydration study in which Jojoba Esters were compared to other petrolatum-alternatives over four hours, and (5) a consumer preference study in which a consumer survey was used to compare Jojoba Esters to a known petrolatum-alternative ingredient.

Materials and Methods

Subjects – All studies were conducted in the Clinical Testing Laboratory at Floratech, Chandler, Arizona. Institutional Review Board approval and a written informed consent from each subject were obtained before any protocol-related procedures were undertaken. All study participants were healthy males and females with normal appearing inner forearm skin, outer leg skin, or hands (as applicable). Upon arriving at the testing facility for each study, subjects acclimated for 30 minutes in a controlled environment with a temperature of 20-22° C and a relative humidity of less than 50%. All studies were carried out in a double-blind, vehicle-controlled, randomized fashion. A total of 71 subjects (55 females and 16 males) ranging in age from 25 to 65 years participated in the studies. All subjects underwent a one to two day “washout” where a commercial soap, supplied to them by the testing lab, was used to wash their forearms, legs, or hands (as applicable). The subjects were instructed not to use any topical products on the test sites during the washout period and the duration of the study.

Test Materials – The vehicle formulation for all studies included the following ingredients (w/w): Water (qs), Glyceryl Stearate (and) PEG-100 Stearate (4.00%), Cetyl Alcohol (3.00%), Phenoxyethanol (and) Methylparaben (and) Ethylparaben (and) Butylparaben (and) Propylparaben (0.80%), Xanthan Gum (0.20%) and Disodium EDTA (0.03%). The test articles included either various Jojoba Esters^a with different melting points, a petrolatum alternative

^a Jojoba Esters were obtained from International Flora Technologies, Ltd. (Chandler, AZ, USA).

[Castor Isostearate Succinate (and) Hydrogenated Castor Oil^b, Canola Oil (and) Zea Mays (Corn) Starch (and) Silica^c, or *Butyrospermum parkii* (Shea Butter)], or Petrolatum,^d in addition to the vehicle formula. Jojoba Esters (*i.e.* INCI name) used in this study were Floraesters[®] 20, mp=45°C; Floraesters 30, mp=50°C; and Floraesters 60, mp=60°C.

Evaluation of Barrier Function (Pre-SLS Treatment) – For Study 1, a total of six, one inch by one inch, test sites were assigned as follows: the vehicle lotion, 2% Floraesters 20, Floraesters 30, or Floraesters 60 in the vehicle lotion, 5% petrolatum in the vehicle lotion (positive control), and untreated skin (negative control). Each test site was demarcated on the inner aspects of the lower arms, located between one inch below the elbow and one inch above the wrist.

Following test site demarcation, baseline TEWL readings (in duplicate) were taken at each site using the Tewameter TM 300 (Courage + Khazaka, Köln, Germany) followed by one application of each test article. At time zero, 20 µl of each test article (vehicle, 2% Floraesters 20, 2% Floraesters 30, 2% Floraesters 60, or 5% petrolatum) was applied to the appropriate skin test site using a one milliliter syringe and spread evenly over the site with a finger cot. For the untreated site, a finger cot was rubbed across the site to mimic the test article application process. After 15 minutes, a 19mm Hill Top Chamber[®] (Hill Top Research Incorporated, St. Petersburg, FL, USA) containing 60 µl of a 0.3% solution (w/w) of SLS^e was placed in the center of each test site. Each chamber was then taped with First Aid Secure- Comfort[®] Soft Tape (Johnson & Johnson Corporation, New Brunswick, NJ) and then overlaid with Coban[®] Self-Adhering Wrap (3M Company, St. Paul, MN). Subjects were then permitted to leave the testing facility and

^b Zenolatium[®] (Zenitech LLC Corporation, Dacula, GA, USA)

^c Vegetatum[®] (Naturanola Health Inc., Winchester, Ontario, Canada)

^d Petrolatum was obtained from International Group, Inc (Toronto, ON, Canada).

^e Sodium lauryl sulfate was obtained from Lubrizol Corporation (Cleveland, OH, USA).

instructed to leave the chambers undisturbed for approximately 12 hours without getting them wet.

Subjects returned to the testing facility, Day 2, and the Hill Top Chambers were removed. The subjects equilibrated for approximately 30 minutes in an environment with a temperature of 21.4-22.2°C and a relative humidity between 40-50% followed by final TEWL readings.

Study 2 was a dose response study conducted in the same manner as Study 1. Five concentrations of Floraesters 60 were evaluated (2%, 1.5%, 1%, 0.5%, and 0.2%). A total of 8 test sites were demarcated: the vehicle lotion, Floraesters 60 at five concentrations in the vehicle lotion, 2% petrolatum in the vehicle lotion (positive control), and untreated skin (negative control).

Evaluation of Barrier Recovery (Post-SLS Treatment) – For Study 3, six approximately one inch by one inch squares were demarcated on the inner aspects of the lower arms of each subject, between one inch below the elbow and one inch above the wrist. These included the vehicle lotion, 2% Floraesters 20, 2% Floraesters 30, or 2% Floraesters 60 in the vehicle lotion, 5% petrolatum in the vehicle lotion (positive control), and untreated skin (negative control).

Following test site demarcation, baseline TEWL measurements were taken at each test site in duplicate using the Tewameter, followed by disruption of the barrier using a solution of 0.3% SLS applied under occlusion in Hill Top Chambers. Subjects were then permitted to leave the testing facility. Approximately 18 hours after SLS application the subjects returned to the testing facility, Day 2, where the Hill Top Chambers were removed. Following a 30 minute acclimation period under controlled environmental conditions, TEWL measurements were taken at each site. Test articles were then applied to each site hourly for a total of three applications. TEWL measurements were repeated one hour after each test article application.

Evaluation of Skin Hydration – For Study 4, the skin hydration study, all subjects had a Corneometer reading of 35 or less on the outer aspect of each lower leg in order to be enrolled into the study.

Once enrolled, four approximately four centimeter by four centimeter squares were demarcated on the outer aspect of each lower leg of each subject, approximately three inches below the knee to approximately three inches above the ankle. Eight test sites were demarcated: six experimental, one negative control (distilled water), and one positive control (10.0% petrolatum in the vehicle). The sites were rotated from the right to left lower outside leg among the subjects as well as from top to bottom of the leg in order to remove any position bias. Baseline Corneometer readings (in triplicate) were taken at each site. Following baseline measurements, one 40 µl application of each test article was made to the designated test site by the clinical staff and spread over the site with a finger cot. Skin hydration measurements were then taken every hour for four hours.

Evaluation of Consumer Preference – For Study 5, the consumer preference study, one 60 µl application of 2% Floraesters 20 or 5% Canola Oil (and) Zea Mays (Corn) Starch (and) Silica, was applied to the back of each hand (one test article per hand) by the clinical staff. The subjects then used the opposite hand to rub each test article into the skin. A preference evaluation containing a list of various skin sensory and formula attributes were given to each subject immediately after application. Each subject was asked to choose which of the two formulas they preferred or to choose “no preference”. The skin attributes that were evaluated included skin moistness, skin suppleness, skin dryness/roughness, and skin smoothness. The formula attributes evaluated were greasiness, whiteness, and stickiness.

Data Analysis

The original data was captured on case report forms and then transferred to a Microsoft[®] Office Excel 2003 (Microsoft Corporation, Redmond, WA, USA) spreadsheet. Data was analyzed using GraphPad InStat 3 (GraphPad Software, Inc., La Jolla, CA).

For Studies 1 and 2 the Mann-Whitney Test (nonparametric, two-tailed p value) was used to determine any statistical significance ($p < 0.05$) using the raw data at baseline and the final evaluation time point for each test article. For Studies 3 and 4, the Kruskal-Wallis Test (nonparametric ANOVA) with the Dunn's Multiple Comparisons Post Test was used to determine any statistical significance ($p < 0.05$) between baseline and each evaluation time point for each test article.

The raw data was also used to calculate the percent improvement in SLS-induced TEWL (relative to the water control) in Studies 1 and 2, the percent barrier recovery from post-insult (untreated) back to baseline (pre-insult, untreated) for each measurement in Study 3, and the percent change in skin hydration from baseline for each evaluation time point for Study 4. In all cases, these changes were averaged for each subject. This data was then analyzed using GraphPad InStat 3. Statistical significance ($p < 0.05$) was determined between the various test articles at each evaluation time point using the Kruskal-Wallis Test (nonparametric ANOVA) with the Dunn's Multiple Comparisons Post Test. Outliers were removed from the mean percent changes using Chauvenet's Criterion for rejecting data.

For the consumer preference study, the original preference questionnaire data was transferred to a Microsoft[®] Office Excel 2003 spreadsheet, preference choices were tallied, and percent preference was calculated.

Results

Barrier Function (Pre-SLS Treatment) – The results of Studies 1 and 2 are depicted in Figures 1 and 2, respectively. In Study 1, the test article containing 2% Floraesters 60 produced the highest percent reduction in SLS-induced TEWL. This was followed by 5% petrolatum, 2% Floraesters 30, and 2% Floraesters 20. All formulas outperformed the vehicle ($p < 0.001$). There were no statistical differences among the test articles containing Jojoba Esters, or between the test articles containing Jojoba Esters and 5% petrolatum.

In Study 2, the dose response curve for Floraesters 60 (Figure 2) revealed that a maximum inhibition of SLS induced TEWL occurred at approximately 1.0% Floraesters 60. Additional increases in the concentration of Floraesters 60 did not produce additional efficacy. The test article containing 2.0% petrolatum inhibited the SLS induced TEWL approximately equal to that of the 1.0% Floraesters 60. The test articles containing 1.0% Floraesters 60, 1.5% Floraesters 60, and 2.0% Floraesters 60 along with the test article containing 2.0% petrolatum produced statistically significant decreases ($p < 0.001$) in TEWL over that of the vehicle.

Figure 1

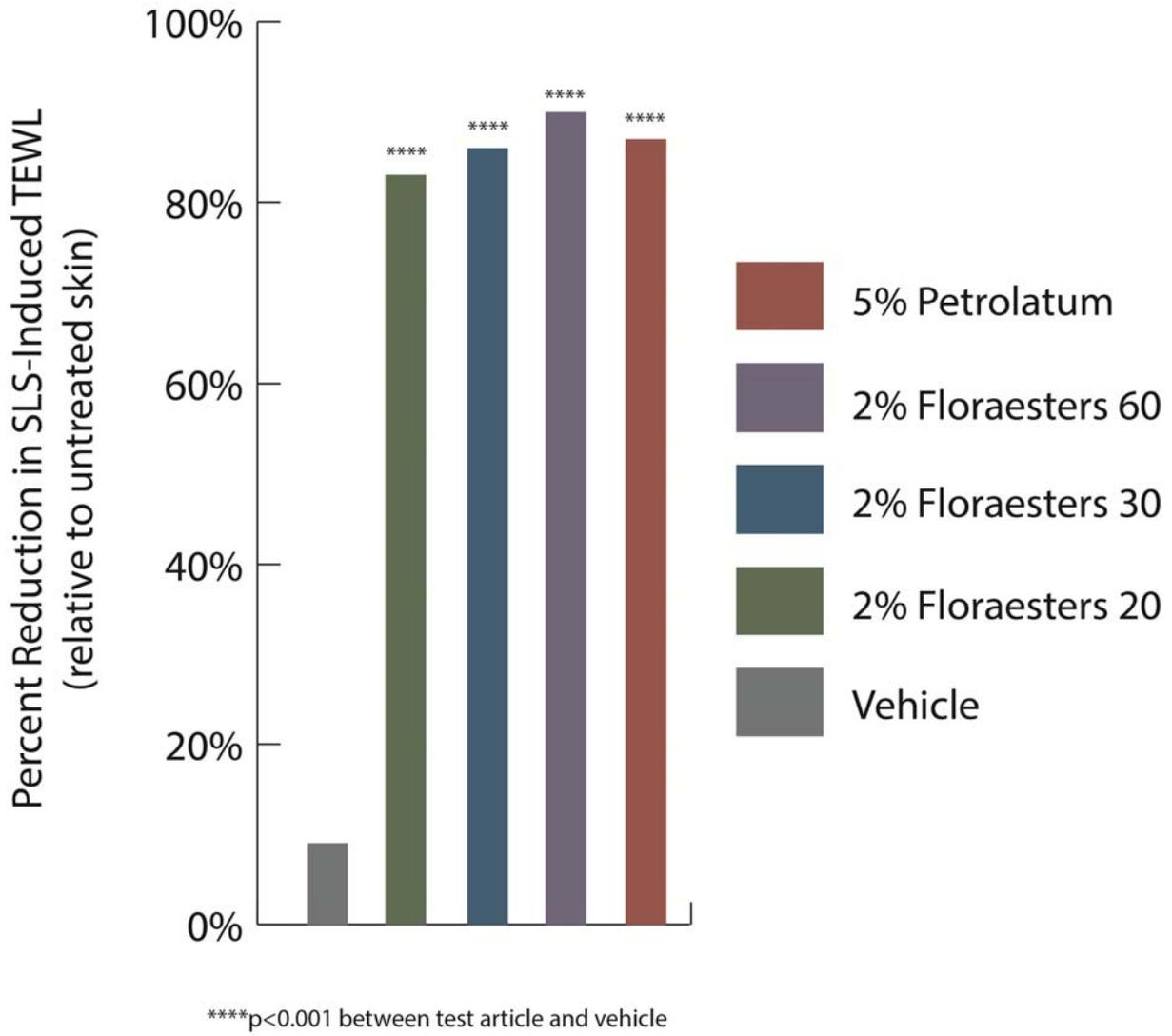
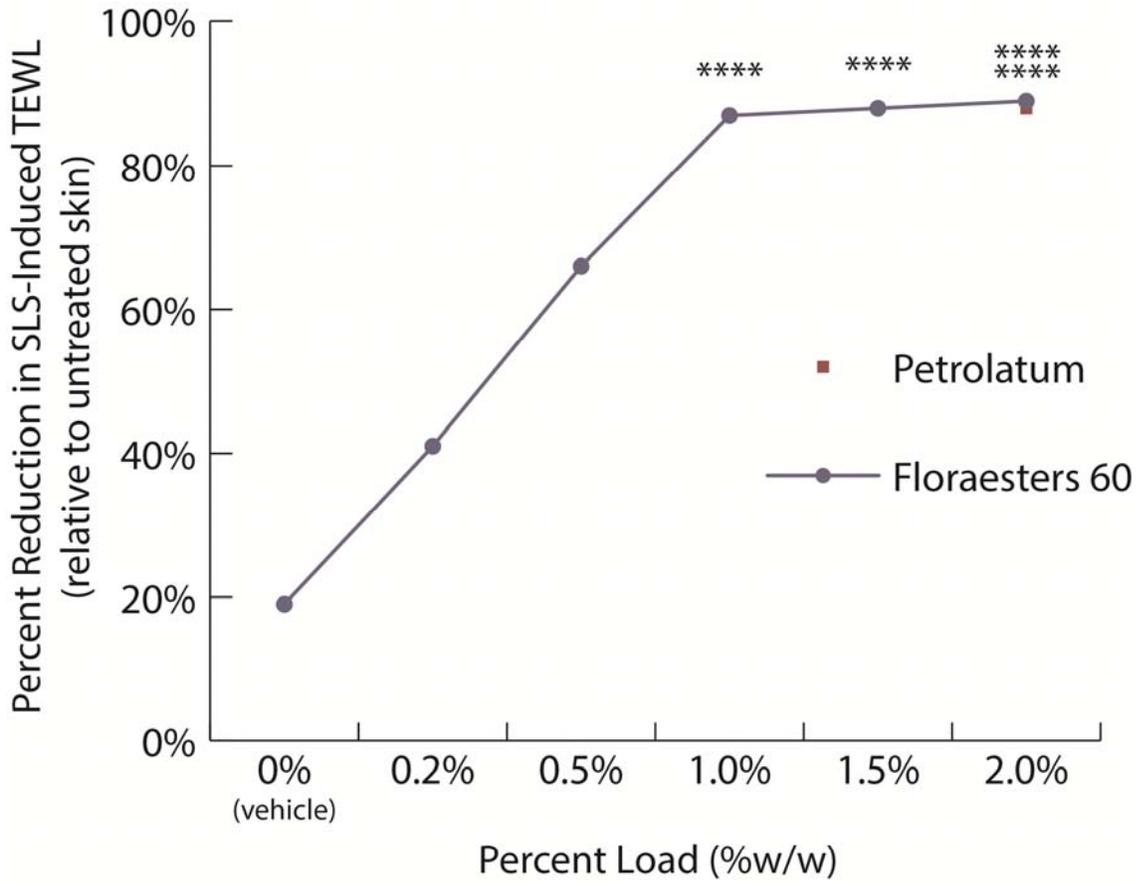


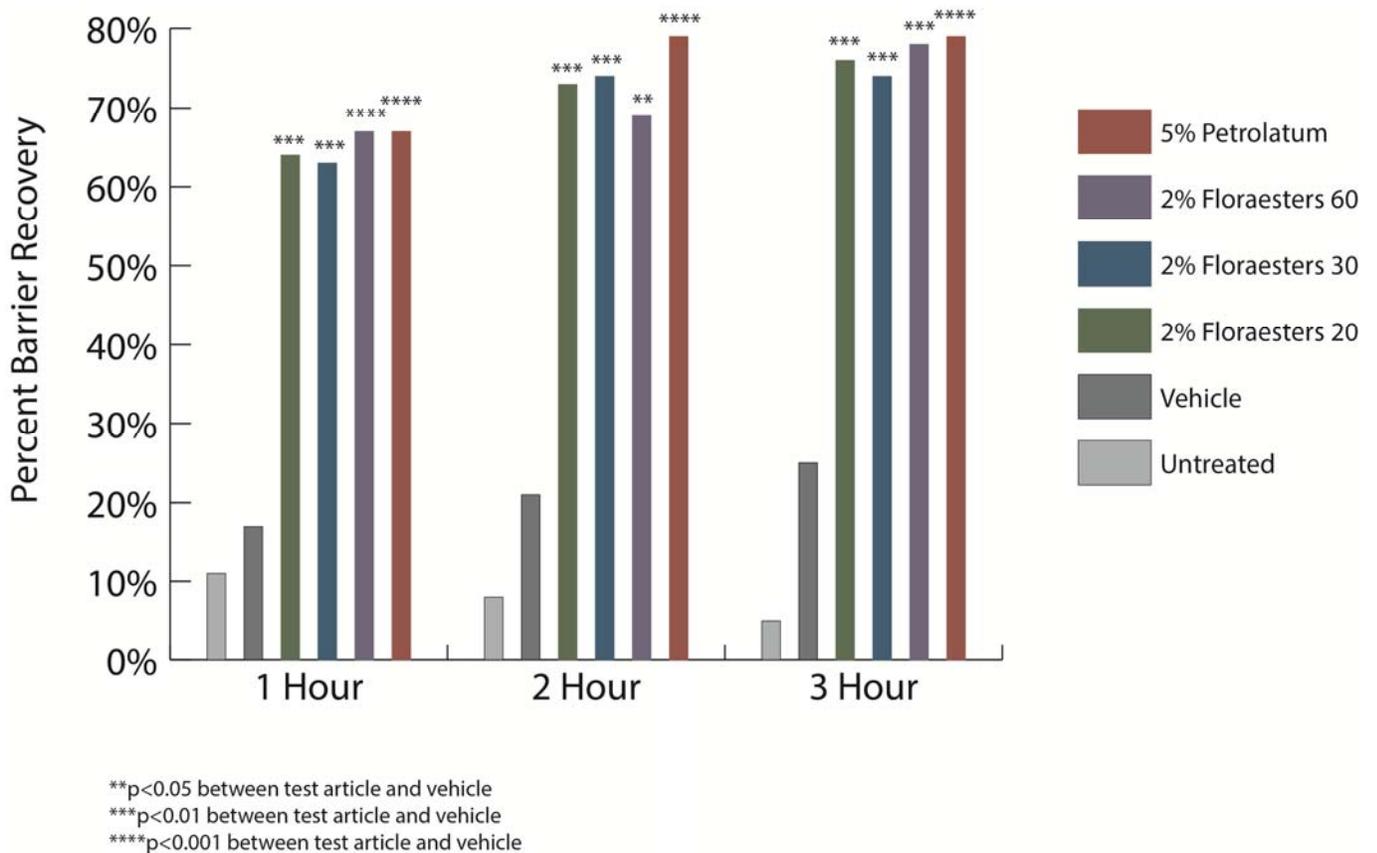
Figure 2



****p<0.001 between test article and vehicle

Barrier Recovery (Post-SLS Treatment) – The results of the skin barrier recovery study, Study 3, are depicted in Figure 3. The test articles containing 2% Floraesters 20, 2% Floraesters 30, and 2% Floraesters 60 produced percent barrier recoveries statistically equivalent ($p < 0.05$) to that of 5% petrolatum after one, two, and three applications. After three applications, the test article containing 5% petrolatum produced the greatest percent barrier recovery. This was followed closely, in order, by the test articles containing 2% Floraesters 60, 2% Floraesters 30, and 2% Floraesters 20. All formulas outperformed the vehicle ($p < 0.05$) at all time points.

Figure 3

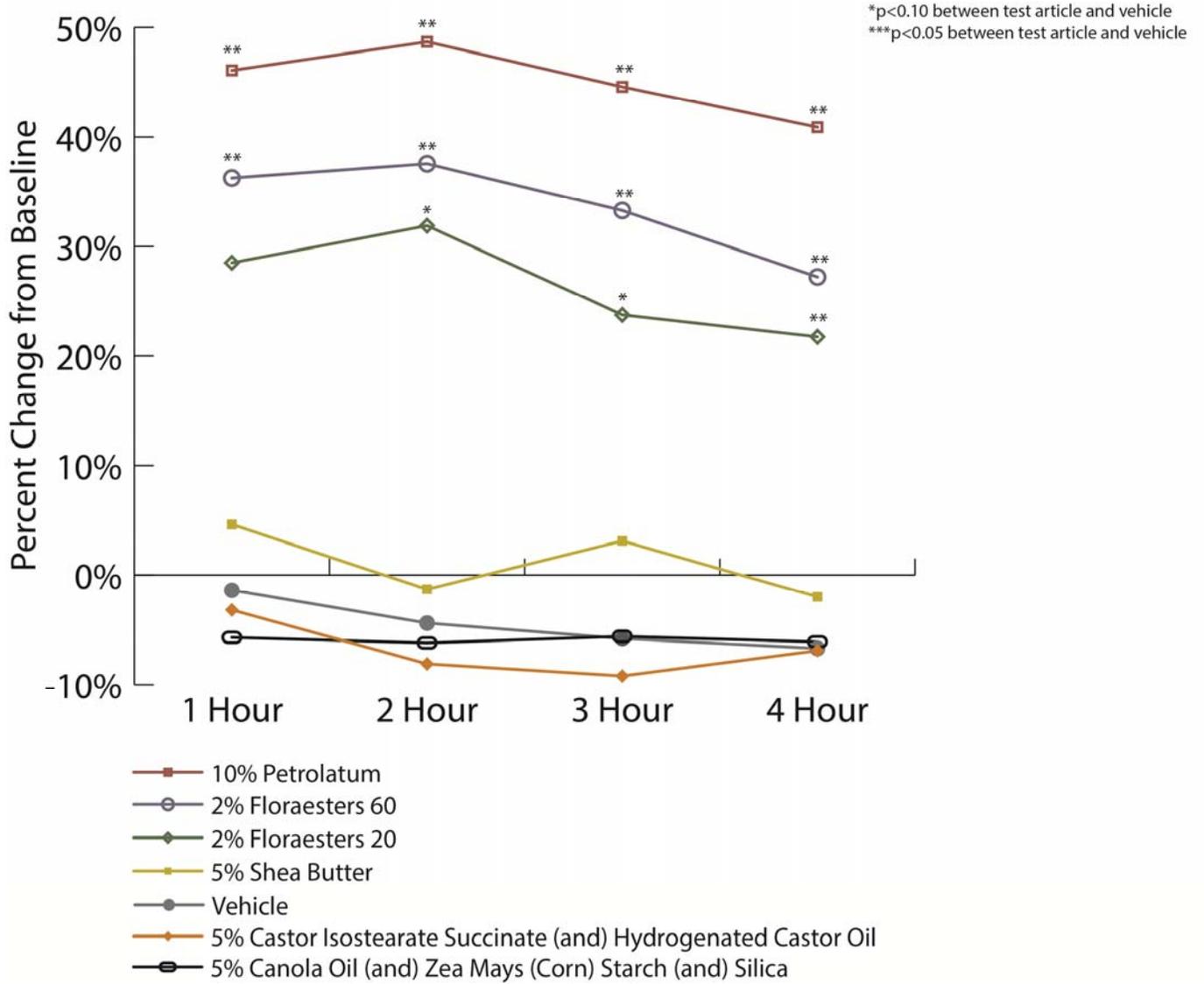


Skin Hydration – The results of the skin hydration study are depicted in Figure 4. The test article containing 2% Floraesters 60 produced higher Corneometer readings than any other test article (with the exception of the positive control, 10% petrolatum) at one, two, three, and four hours post test article application, with a peak increase in skin hydration at the two hour evaluation time point ($p < 0.05$). In addition, this formula outperformed the vehicle, Castor Isostearate Succinate (and) Hydrogenated Castor Oil, and Canola Oil (and) Zea Mays (Corn) Starch (and) Silica at every evaluation time point ($p < 0.05$).

Another test article, 2% Floraesters 20, produced a peak increase in Corneometer readings at the two hour evaluation time point. This formula outperformed the vehicle, Castor Isostearate Succinate (and) Hydrogenated Castor Oil, and Canola Oil (and) Zea Mays (Corn) Starch (and) Silica with directional significance ($p < 0.10$) at most evaluation time points.

The three petrolatum-alternatives: Castor Isostearate Succinate (and) Hydrogenated Castor Oil (5.00%), Canola Oil (and) Zea Mays (Corn) Starch (and) Silica (5.00%) and *Butyrospermum parkii* (Shea Butter) (5.00%) produced minimal increases in skin hydration and did not achieve statistical significance from baseline at any evaluation time point.

Figure 4

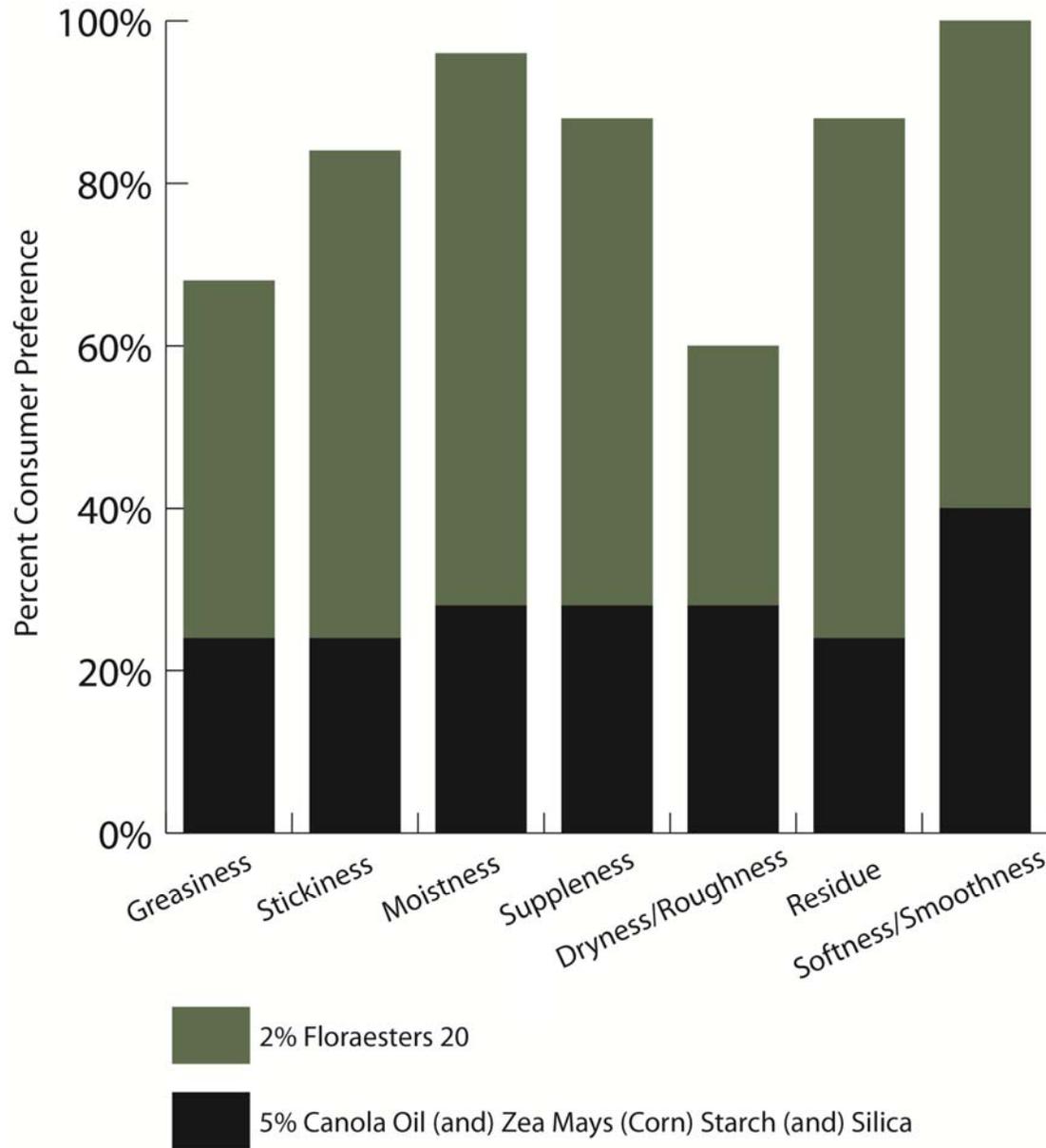


Consumer Preference – Sensory evaluations are often difficult to capture and do not always agree with bio-instrumental results. However, sensory evaluations are very important because consumers may ultimately decide which products to buy based on preferences rather than objective scientific measurements.

Two test articles from the skin hydration study were compared in a consumer preference study: 2% Floraesters 20 was compared to 5% Canola Oil (and) Zea Mays (Corn) Starch (and) Silica. Data from the preference study are depicted in Figure 5. The test article with 2% Floraesters 20 was preferred over the formula containing the marketed petrolatum-alternative ingredient by 68% of the subjects for moistness immediately after test article application. This supports the results of the skin hydration study which showed that 2% Floraesters 20 hydrated the skin better than the test article with this same marketed petrolatum-alternative ingredient.

More than twice as many consumers indicated that the formula containing 2% Floraesters 20 left the skin less sticky and less greasy than the lotion containing the marketed petrolatum-alternative ingredient. In addition, 68% of the subjects also indicated that the test article containing 2% Floraesters 20 left the skin more supple. The test article with 2% Floraesters 20 was also seen as less greasy than the test article containing the marketed petrolatum-alternative ingredient. In addition, the lotion containing 2% Floraesters 20 was perceived to produce more smooth/soft skin, less dry/rough skin, and left less of a residue on the skin than the lotion containing the marketed petrolatum-alternative ingredient.

Figure 5



CONCLUSIONS

The data presented in this paper show that specific Jojoba Esters with distinct melting points (*i.e.*, Floraesters 20, 30, and 60) have the capability of reducing damage to the skin barrier, as measured by TEWL, in a dose dependent manner. The data also suggests that these esters can

accelerate recovery of the skin barrier damaged by SLS, as well as provide the skin with the hydration necessary to maintain a healthy barrier. In addition, the barrier and skin hydration activity of these natural esters is similar to the activity of petrolatum.

It is not surprising that Jojoba Esters have beneficial effects on the skin. The predecessor of these esters, jojoba oil, has been used in folk remedies for sunburn, chaffed skin, and wounds.¹⁷ In 1995, Habashy *et.al.* reported the anti-inflammatory effects of jojoba liquid wax in a number of experimental models.¹⁸ More recently we have reported that another closely related class of Jojoba Esters (*i.e.*, hydrolyzed Jojoba Esters) were able to decrease TEWL and increase skin hydration in the presence of glycerin in patients with hand eczema.¹⁹ That same study also showed inhibition of staining for filaggrin and IL-6 by hydrolyzed Jojoba Esters.¹⁹

The mechanism of action of Floraesters 20, Floraesters 30, and Floraesters 60 has not been identified. The possibility that Jojoba Esters are acting like an occlusive agent similar to petrolatum is a definite possibility. However, another possibility is that these esters may influence cellular events associated with barrier function, thereby producing protective and restorative properties to the skin. Future research will explore the mechanism behind these biologically active molecules.

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