

Skin Brightening Effects of Ascorbic Acid with ACTISOLV™ Integration: A Longitudinal Analysis Using Repeated Measures ANOVA

Dita Amelia^{1*}, Suliyanto², Arinda Mahadesyawardani³, Nur Azizah⁴, Jovansha Ariyawan⁵, R. Arya Januarta Adiputra⁶, Irsyad Yoga Adyatma⁷

¹²³⁴⁵⁶⁷ Department of Mathematics, Universitas Airlangga, Indonesia

Email: dita.amelia@fst.unair.ac.id^{1*}, suliyanto@fst.unair.ac.id², arinda.mahadesyawardani-2022@fst.unair.ac.id³, nur.azizah-2022@fst.unair.ac.id⁴, jovansha.ariyawan-2022@fst.unair.ac.id⁵, r.arya.januarta.adiputra-2022@fst.unair.ac.id⁶, irsyad.yoga.adyatma-2022@fst.unair.ac.id⁷

*Corresponding author

Abstract

Ascorbic Acid is a well-established active ingredient recognized for its efficacy in skin brightening and its antioxidant properties. However, its low chemical stability in cosmetic formulations remains a significant limitation. In response to this challenge, the patent-pending ACTISOLV™ formulation technology has been developed to enhance the stability of Ascorbic Acid, improving its resistance to oxidation and effectiveness in inhibiting melanin production. This study contributes to the advancement of sustainable cosmetic science through the development of more stable, eco-friendly, and sustainable skincare products. This study aimed to evaluate the longitudinal effects of a cream containing 20% Ascorbic Acid formulated with ACTISOLV™ on skin brightness. This study used longitudinal experimental data analyzed by Repeated Measures ANOVA. The results demonstrated a statistically significant increase in skin brightness over time ($p\text{-value} = 0.000 < 0.05$). Posthoc analysis revealed that skin brightness at weeks 6 and 12 differed significantly from baseline. However, no significant difference was found between the control and treatment groups and no significant interaction was observed between time and treatment group. These findings suggest that the observed improvement in skin brightness occurred similarly in both groups, indicating no additional effect attributable to the test formulation.

Keywords: Ascorbic acid, Longitudinal Data, Repeated Measures ANOVA, Skin Brightening.

1. INTRODUCTION

One of the active ingredients that has been proven effective in brightening and firming the skin is Ascorbic Acid (Vitamin C). As a powerful antioxidant, Ascorbic Acid can neutralize UV radiation and stimulate collagen synthesis, making it effective in brightening and tightening the skin.



JURNAL MATEMATIKA, STATISTIKA DAN KOMPUTASI
Dita Amelia, Suliyanto, Arinda Mahadesyawardani, Nur Azizah, Jovansha
Ariyawan, R. Arya Januarta Adiputra, Irsyad Yoga Adyatma

However, the main challenge in its use in cosmetic products is its instability, which causes rapid oxidation and decreased efficacy during storage [14]. To address this issue, formulation innovations such as Ascorbic acid-2-glucoside (AA2G) and ACTISOLV™ technology have been developed, where AA2G as a stable form of vitamin C will maintain antioxidant bioactivity and tyrosinase inhibition, thereby increasing stability against oxidative degradation [5]. This mechanism makes AA2G effective in reducing hyperpigmentation, thereby preventing melanin accumulation [19]. This technology opens up new opportunities in the cosmetics industry by introducing more effective, stable, and innovative skin care products that are more sustainable and environmentally friendly, in line with the principles of SDGs point 3, Good Health and Well-being, and SDGs point 12, Responsible Consumption and Production.

The appropriate statistical approach is essential in clinical trials assessing the skin-brightening effects of Ascorbic Acid using ACTISOLV™ technology. Clinical trials often require repeated or longitudinal observations to assess changes in skin lightening levels over time. ANOVA is a simple approach that can be used for longitudinal data analysis, where, in principle, its use is technically straightforward but may encounter issues if standard assumptions such as balance and homogeneity of variance are not met [4]. In this case, as with longitudinal data, the use of ANOVA can ignore variability within subjects, leading to errors in inference [7]. Repeated Measures ANOVA is an extension of simple ANOVA designed for longitudinal data. This method takes into account the correlation between measurements taken on the same individual at different times or under different conditions [12]. By treating each individual as their own control, Repeated Measures ANOVA reduces between-subject variability [8], thereby increasing sensitivity in detecting changes over time. The application of Repeated Measures ANOVA requires special attention to its assumptions, such as data balance and sphericity (constant variance across all time points), to obtain unbiased results and proper interpretation [12].

Research conducted by Shrotri et al. (2025) [17] used Repeated Measure ANOVA to analyze changes in gene expression in psoriasis patients after various therapeutic interventions using Gene Set Variation Analysis. In the study, it was found that gene expression related to inflammation, keratinocyte proliferation, and immunological pathways underwent significant changes after treatment, enabling the identification of distinct patterns of gene expression changes between responders and non-responders to therapy. A study with the same method and scope, focusing on skin health, was also conducted by Chilicka et al. (2022) [3], which demonstrated that Repeated Measures ANOVA can be effectively used to analyze longitudinal changes in skin condition following specific treatments. The study evaluated the effectiveness of Oxybrasion in reducing acne in young women. The study compared the treatment group with the placebo group. The results showed that only the treatment group experienced a reduction in acne, improved skin hydration, and reduced sebum production, while the placebo group did not show significant changes.

Based on previous studies, a similar approach can be applied in this study which aims to test the difference in average skin brightness levels of Ascorbic Acid with ACTISOLV™ technology based on treatment and time factors. The novelty of this study lies in the use of the latest clinical data which is the integration of Ascorbic Acid in the ACTISOLV™ formulation. Although previous studies have investigated the efficacy and stability of Ascorbic Acid, research on the long-term stability of Ascorbic Acid in the ACTISOLV™ formulation remains limited. Beyond formulation aspects, this study further reinforces the relevance of using Repeated Measures ANOVA in studies focused on the longitudinal efficacy of skin care products. Thus, this research provides a robust scientific analytical approach for assessing the longitudinal effectiveness of skincare products, aligning with the needs of both the industry and modern consumers.

JURNAL MATEMATIKA, STATISTIKA DAN KOMPUTASI
Dita Amelia, Suliyanto, Arinda Mahadesyawardani, Nur Azizah, Jovansha
Ariyawan, R. Arya Januarta Adiputra, Irsyad Yoga Adyatma

2. METHODS

2.1 Longitudinal ANOVA

Longitudinal data are collected from the same units of analysis at two or more time points to study changes in a phenomenon over time. This type of data allows researchers to compare an individual across time [18]. Analysis of Variance (ANOVA) is a statistical method used to test for differences in means among two or more groups in a study. This method was first introduced by Ronald A. Fisher and has become a fundamental technique in the analysis of experimental data [11]. ANOVA has limitations when analyzing data that involve repeated measurements on the same subjects. In cases such as longitudinal data, the use of standard ANOVA can ignore within-subject variability, potentially leading to erroneous inferences [7]. To address this issue, Repeated Measures ANOVA (RM-ANOVA) is employed. This method is specifically designed for data involving the same subjects under several different conditions or time points. This technique is highly useful for testing mean differences across conditions in designs involving repeated measurements, such as longitudinal experiments or pre-post tests. Because the same subjects are used in every condition, RM-ANOVA controls for between-individual variability, resulting in a more sensitive test compared to a standard ANOVA.

The statistical model for RM-ANOVA considers the fixed effects of treatment or time, the random effects of individuals, and the error. The general RM-ANOVA model for cases without treatment, or a randomized block ANOVA, is written in Equation (2.1) [2].

$$Y_{ij} = \mu + \pi_i + \alpha_j + \varepsilon_{ij} \quad (2.1)$$

where,

- Y_{ij} : measurement value for the i -th subject at the j -th time
- μ : overall mean
- α_j : effect of time or the treatment effect at time j
- π_i : random effect of the i -th subject
- ε_{ij} : is the error effect associated with subject i at time j

The random components are also assumed to have the distributions $\pi_i \sim N(0, \sigma_\pi^2)$, where σ_π^2 is the between-individual variance, and $\varepsilon_{ij} \sim N(0, \sigma_\varepsilon^2)$, where σ_ε^2 is the within-individual variance. The model commonly used when treatment is included in the data is called the split-plots ANOVA, written as follows.

$$Y_{hij} = \mu + \beta_h + \alpha_j + (\beta\alpha)_{hj} + \pi_{i(h)} + \varepsilon_{hij} \quad (2.2)$$

where,

- μ : overall mean
- β_h : effect of the h -th group with $\sum_{h=1}^H \beta_h = 0$
- α_j : effect of time j with $\sum_{j=1}^J \alpha_j = 0$
- $(\beta\alpha)_{hj}$: interaction effect between time and group with $\sum_{h=1}^H \sum_{j=1}^J (\beta\alpha)_{hj} = 0$
- $\pi_{i(h)}$: effect of individual i in group h at time j
- ε_{hij} : error for individual i in group h at time j

The random components are also assumed to have the distributions $\pi_{i(h)} \sim N(0, \sigma_\pi^2)$ where σ_π^2 is the between-individual variance, and $\varepsilon_{ij} \sim N(0, \sigma_\varepsilon^2)$ where σ_ε^2 is the within-individual variance. This implicitly assumes compound symmetry for the variance-covariance matrix $Var(y_i)$ [4].

Compound symmetry, shown in (2.4), is important in repeated measures analysis because it states that observations within one individual are correlated, but observations between individuals

JURNAL MATEMATIKA, STATISTIKA DAN KOMPUTASI
Dita Amelia, Suliyanto, Arinda Mahadesyawardani, Nur Azizah, Jovansha
Ariyawan, R. Arya Januarta Adiputra, Irsyad Yoga Adyatma

remain independent. The relationship between observations within an individual is defined as the correlation, written in the Equation (2.3).

$$\text{Corr}(y_{ij}, y_{ik}) = \frac{\sigma_{\pi}^2}{\sigma_{\pi}^2 + \sigma_{\varepsilon}^2} \quad (2.3)$$

where $\sigma_{\pi}^2 + \sigma_{\varepsilon}^2$ represents the homogenous variance of each observation for every j , derived from the expansion of $\text{Var}(y_{ij}) = \text{Var}(\mu + \pi_i + \alpha_j + \varepsilon_{ij})$ where $\sum_{j=1}^J \alpha_j = 0$. The covariance between the same individuals at different time points is written as $\text{Cov}(y_{ij}, y_{ik}) = \sigma_{\pi}^2$. Thus, the structure of the variance-covariance matrix for y_i for repeated observations within a single individual is as follows [4].

$$\begin{bmatrix} \sigma_{\pi}^2 + \sigma_{\varepsilon}^2 & \sigma_{\pi}^2 & \cdots & \sigma_{\pi}^2 \\ \sigma_{\pi}^2 & \sigma_{\pi}^2 + \sigma_{\varepsilon}^2 & \cdots & \sigma_{\pi}^2 \\ \vdots & \vdots & \ddots & \vdots \\ \sigma_{\pi}^2 & \sigma_{\pi}^2 & \cdots & \sigma_{\pi}^2 + \sigma_{\varepsilon}^2 \end{bmatrix} \quad (2.4)$$

RM-ANOVA has several important assumptions, such as data normality and sphericity (equality of variances of the differences between conditions). One of the greatest challenges in using RM-ANOVA is the violation of the sphericity assumption. If this occurs, it can lead to incorrect conclusions unless a statistical correction, such as the Greenhouse-Geisser or Huynh-Feldt correction, is applied. According to research [2], RM-ANOVA is quite robust to violations of the normality assumption, especially when the sample size is sufficiently large. However, if the sample size is small and the data are highly non-normal (e.g., highly skewed or possessing high kurtosis), the risk of a Type I Error increases, and the test's power may decrease. Therefore, researchers must consider the data distribution and sample size when employing this method.

2.2 Research Data

The type of research used is quantitative research with the data used is secondary data obtained from the study conducted by Sass et al. (2023) [16], with the dataset publicly available on data.mendeley.com. This dataset includes clinical trial results of a new patented skincare technology, measuring Ascorbic Acid levels and its skin-brightening effects. The 12-week trial involved 35 patients divided into a control group (18 patients using a placebo cream) and a test group (17 patients using a 20% Ascorbic Acid cream with ACTISOLV™). Skin brightness was assessed at weeks 0, 2, 6, and 12 using the Konica Minolta Chromameter CR-400. This study involved a series of statistical analyses aimed at evaluating the effectiveness of a cream containing 20% Ascorbic Acid and ACTISOLV™ in improving skin brightness using the Longitudinal ANOVA method with Repeated Measures.

2.3 Data Analysis Procedure

- (1) Conducting descriptive statistics and visualizations such as mean structure, variance structure, scatterplots, and correlation patterns from week 0 to week 12 to better understand the longitudinal data trends.
- (2) Assumption Testing for Repeated Measures Design

a. Sphericity assumptions

The data were tested using Mauchly's Test of Sphericity to determine whether the variances between groups are equal [1]. In repeated measures ANOVA, it is assumed that the

JURNAL MATEMATIKA, STATISTIKA DAN KOMPUTASI
Dita Amelia, Suliyanto, Arinda Mahadesyawardani, Nur Azizah, Jovansha
Ariyawan, R. Arya Januarta Adiputra, Irsyad Yoga Adyatma

covariance matrix of repeated measurements follows a specific pattern called “sphericity” or “spherical matrix.” Therefore, the Mauchly Test of Sphericity must be performed to test whether this assumption is met, both in the analysis of main effects and interactions [10]. If this assumption is not met, the ANOVA results may be biased and increase the likelihood of type 1 errors. The hypothesis of the Mauchly’s Test of Sphericity are stated as follows [2].

H_0 : The variance of the differences between all combinations of groups (levels) are equal (sphericity is assumed)

H_1 : The variance of the differences between all combinations of groups (levels) are not equal (sphericity is violated)

The test statistics from Mauchly's Test of Sphericity using Equation (2.5).

$$W = \frac{|T|}{\frac{tr(T)}{p} \times d} \quad (2.5)$$

where $T = M' \hat{\Sigma} M$, with $\hat{\Sigma}$ being the sample covariance matrix. While Σ is a covariance

matrix of $p \times p$ data, with $\Sigma = \sigma^2 \begin{pmatrix} 1 & \rho & \dots & \rho \\ \rho & 1 & \dots & \rho \\ \vdots & \vdots & \ddots & \vdots \\ \rho & \rho & \dots & 1 \end{pmatrix}$, and σ^2 is the population variance of

each variable. M is a contrast matrix of size $d \times p$, where $d = p - 1$.

The significance of the W statistic is assessed using a chi-square is given in Equation (2.6)

$$C = -(n - r) \log(W) \cdot D \quad (2.6)$$

with $df = \frac{p(p-1)}{2} - 1$, and D as a correction factor.

The critical region is rejected the null hypothesis if $C > C_{\alpha, df}$ or if the p-value is less than alpha 5%. If the sphericity assumption is not met, corrections such as the Greenhouse-Geisser adjustment are needed to correct the degrees of freedom [6].

b. Normality of residuals

The normality assumption of residuals is tested using the Kolmogorov–Smirnov test to determine whether the residuals follow a normal distribution [12]. The hypothesis for the residual normality test are formulated as follows.

H_0 : The residuals follow a normal distribution

H_1 : The residuals do not follow a normal distribution

The Kolmogorov–Smirnov test statistic is given in Equation (2.7)

$$D = \max_x |F_n(x) - F(x)| \quad (2.7)$$

The decision rule is to reject the null hypothesis if the test statistic Kolmogorov-Smirnov $D > D_{N, \alpha}$ or p-value < 0.05 .

c. Homogeneity of variance

The assumption of homogeneity of variances can be tested using Levene's test, which evaluates whether the variances of the treatment and control groups are equal [20]. The hypothesis are formulated as follows.

JURNAL MATEMATIKA, STATISTIKA DAN KOMPUTASI
Dita Amelia, Suliyanto, Arinda Mahadesyawardani, Nur Azizah, Jovansha
Ariyawan, R. Arya Januarta Adiputra, Irsyad Yoga Adyatma

$H_0 : \sigma_1^2 = \sigma_2^2 = \dots = \sigma_k^2$ or the variances across groups are equal

$H_1 : There is at least one $\sigma_i^2 \neq \sigma_j^2$ or the variances across groups are different$

The test statistic used to assess the homogeneity of variances among groups using Equation (2.8).

$$W = \frac{(n - k) \sum_{i=1}^k n_i (\bar{Z}_i - \bar{Z}_{..})^2}{(k - 1) \sum_{i=1}^k \sum_{j=1}^{n_i} (\bar{Z}_{ij} - \bar{Z}_{..})^2} \quad (2.8)$$

where

n : total number of observations

k : number of treatment groups

n_i : number of replications (sum of sample sizes across groups)

\bar{Z}_i : mean of the residuals in group i

$\bar{Z}_{..}$: overall mean of the residuals across all groups

\bar{Z}_{ij} : absolute deviation (residual) of observation j in group i from the group mean

The rejection region H_0 if the test statistic $W > F_{(\alpha; k-1; n-k)}$ or p-value < 0.05 .

(3) Modelling Skin Brightness Using Repeated Measures Design

a. Main effect of time

Hypothesis:

$H_0 : \alpha_1 = \alpha_2 = \alpha_3 = \alpha_4$ (There is no significant difference in the mean skin brightness levels across different observation times)

$H_1 : There is at least one ij where $\alpha_i \neq \alpha_j$ (There is a significant difference in the mean skin brightness levels across different observation times)$

b. Main effect of treatment

Hypothesis:

$H_0 : \beta_1 = \beta_2$ (There is no significant difference in the mean skin brightness levels across different treatments).

$H_1 : There is at least one ij where $\beta_i \neq \beta_j$ (There is a significant difference in the mean skin brightness levels across different treatments)$

c. Interaction between time and treatment

Hypothesis:

$H_0 : (\alpha\beta)_{11} = (\alpha\beta)_{12} = \dots = (\alpha\beta)_{24} = 0$ (There is no significant interaction effect between observation time and treatment on mean skin brightness levels)

$H_1 : There is at least one ij where $(\alpha\beta)_{ij} \neq 0$ (There is no significant interaction effect between observation time and treatment on mean skin brightness levels)$

(4) Post hoc analysis if any main effects or interactions showed significant results.

A post-hoc analysis using Tukey's Honest Significant Difference (HSD) test was conducted to determine the specific time points where the differences occurred. Tukey HSD works by comparing all pairs of means $(\mu_i - \mu_j)$ between groups and adjusting the significance level with the aim of keeping the family-wise error rate stable [9]. Tukey's HSD is regarded as one of the most precise and effective techniques, especially when the assumption of variance homogeneity is met and sample sizes are fairly balanced [13]. The hypothesis are formulated as follows.

JURNAL MATEMATIKA, STATISTIKA DAN KOMPUTASI
Dita Amelia, Suliyanto, Arinda Mahadesyawardani, Nur Azizah, Jovansha
Ariyawan, R. Arya Januarta Adiputra, Irsyad Yoga Adyatma

$H_0 : \mu_1 = \mu_2 = \mu_3 = \mu_4$ (All pairwise group means are equal or there are no significant differences between any of the groups)

H_1 : There is at least one ij where $\mu_i \neq \mu_j$ (At least one pair of group means differs significantly)

The test statistics from Tukey's HSD using Equation (2.9) [15].

$$q_s = \frac{|Y_A - Y_B|}{SE} \quad (2.9)$$

Where Y_A and Y_B are two means being compared and SE is the standard error for the sum of the means. The rejection region H_0 if the test statistic $q > q(\alpha; k; df_{Error})$ or p-value < 0.05 .

(5) Interpretation

The final step involved interpreting the results regarding the effects of time, treatment, and their interaction. The results were then discussed further by connecting the statistical findings to biological context, particularly explaining the role of Ascorbic Acid in combination with ACTISOLV™ technology in enhancing skin brightness.

3. RESULT

Exploratory individual profile plot of 35 patients, as shown in Figure 1, illustrates the pattern of skin brightness changes for each subject from week 0 to week 12, with the blue lines representing the treatment group (cream with 20% Ascorbic Acid + ACTISOLV™) and the pink lines representing the control group (cream without the active formula).

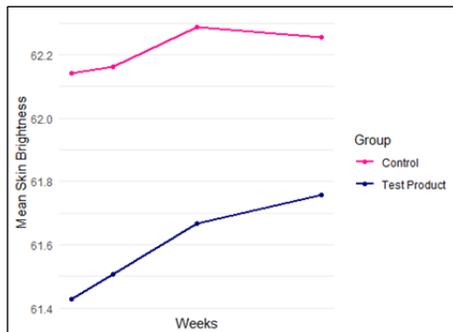


Figure 3.1 Mean Structure by Group

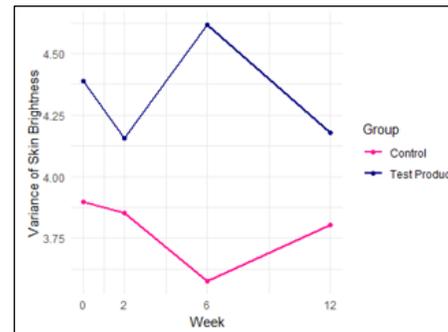


Figure 3.2 Variance Structure by Group

Based on Figure (3.1), the treatment group showed a consistent increase in skin brightness from week 0 to week 12. In contrast, the control group exhibited a slight improvement until week 6, followed by a minor decline at week 12. This pattern suggests that, on average, the test cream had a more sustained brightening effect. Figure (3.2) shows the variance structure between groups: the treatment group had higher and more fluctuating variance, indicating varied individual responses showed substantial improvement, others only slight. Meanwhile, the control group displayed lower variance, suggesting more uniform but minimal changes across individuals.

JURNAL MATEMATIKA, STATISTIKA DAN KOMPUTASI
Dita Amelia, Suliyanto, Arinda Mahadesyawardani, Nur Azizah, Jovansha
Ariyawan, R. Arya Januarta Adiputra, Irsyad Yoga Adyatma

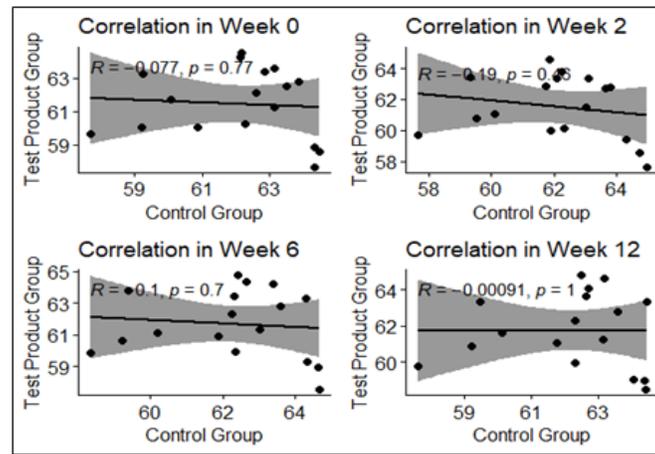


Figure 3.3 Scatterplot and Time Correlation

As shown in Figure (3.3), the scatterplot matrix below the diagonal reveals a strong linear relationship between time points, with data points forming nearly straight lines. This indicates that skin brightness remained stable and highly correlated over time. The consistently high Pearson correlation coefficients further support the conclusion that changes in skin brightness were relatively consistent within individuals throughout the observation period.

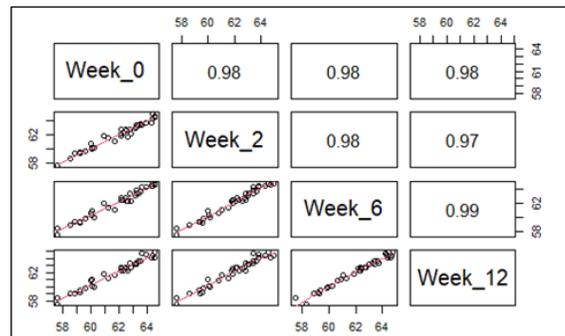


Figure 3.4 Group Correlation Week 0 to 12

As shown in Figure (3.4), the correlation analysis between groups indicates no significant relationship in skin brightness between the control and treatment groups at any observation week. Pearson correlation coefficients (R) ranged from -0.70 to 0.35 with p -values > 0.05 , indicating weak and statistically insignificant associations. Even at week 12, despite a relatively strong negative correlation ($R = -0.70$), the p -value of 0.19 confirms non-significance.

To further examine the effect of Asorbic Acid with ACTISOLV™ on skin brightness, a Repeated Measures ANOVA was conducted on longitudinal data from weeks 0, 2, 6, and 12. This parametric method begins with assumption testing, including the sphericity assumption of the variance-covariance matrix. At a significance level of 0.05 , the null hypothesis is rejected when the p -value $< \alpha$.

JURNAL MATEMATIKA, STATISTIKA DAN KOMPUTASI
Dita Amelia, Suliyanto, Arinda Mahadesyawardani, Nur Azizah, Jovansha
Ariyawan, R. Arya Januarta Adiputra, Irsyad Yoga Adyatma

Table 3.1 Sphericity Test

Within Subject Effect	Test Statistic W	Sig.
Weeks	0.77788	0.15818

The Test of Sphericity in Table (3.1) shows a significance value of 0.15818, which is greater than 0.05. Therefore, we fail to reject the null hypothesis, indicating that the assumption of sphericity is met. This means the variances of the differences between group pairs are equal, and no correction (such as Greenhouse-Geisser) is needed in the Repeated Measures ANOVA.

In Repeated Measures ANOVA, it is assumed that residuals are normally distributed. Using the Kolmogorov–Smirnov test, a p-value of 0.3214 (> 0.05) was obtained, indicating failure to reject the null hypothesis and confirming normality. Additionally, ANOVA assumes equal population variances. Homogeneity was tested using Levene’s Test, with a significance threshold of 0.05; the null hypothesis is rejected if p-value $< \alpha$.

Table 3.2 Levene’s Test of Equality of Error Variances

Weeks	P-Value
0	0.149
2	0.070
6	0.254
12	0.172

Based on the output of the Levene’s Test for homogeneity in Table (3.2), all p-values were greater than 0.05. Therefore, the decision is to fail to reject the null hypothesis, indicating that the residual variances between groups are equal or homogeneous.

After meeting all assumption tests, the analysis proceeded to the significance testing of the Repeated Measures ANOVA. The use of the repeated measures factor aims to determine whether there are significant differences across multiple repeated measurements of the research variable.

Table 3.3 Repeated-Measures ANOVA Test Result

Source of Variation	Sum of Square	df	Residual SS	Residual df	F-value	p-value
Intercept	535998	1	527.60	33	33525.3546	0.000000
Group	14	1	527.60	33	0.8486	0.363631
Weeks	1	3	7.03	99	5.6597	0.001277
Group*Weeks	0	3	7.03	99	1.0201	0.387147

At a significance level of 0.05, the null hypothesis is rejected when the p-value $< \alpha$. Based on the test results in Table 3.3, the hypothesis are outlined as follows.

(1) Hypothesis for testing the main effect by time

Based on the test results, the significance value for the time factor (weeks) is $0.001277 < 0.05$. Therefore, H_0 is rejected, indicating that there is a difference in the average observation time on skin brightness level. Since the result is significant, further analysis such as a post-hoc Tukey HSD test can be conducted to identify which specific time pairs differ significantly.

(2) Hypothesis for testing the main effect by group

Based on the results, the the significance value for the group factor is $0.363631 > 0.05$. Therefore, we fail to reject H_0 , indicating no difference in the average skin brightness level between treatment groups. This means the change in skin brightness cannot be attributed to the treatment, as the average change occurred in both the control and test groups.

JURNAL MATEMATIKA, STATISTIKA DAN KOMPUTASI
Dita Amelia, Suliyanto, Arinda Mahadesyawardani, Nur Azizah, Jovansha
Ariyawan, R. Arya Januarta Adiputra, Irsyad Yoga Adyatma

(3) Hypothesis for testing the interaction effect of time and group

Based on the test results, the significance value for the interaction between group and time was $0.387147 > 0.05$. Therefore, we failed to reject H_0 , which indicates there is no mean difference due to the interaction between time and group on skin brightness. This means the pattern of change in brightness over time was similar in the control group and the test group.

Although the Repeated Measures ANOVA results for hypothesis (1) show that time had a significant effect on increasing skin brightness, the improvement occurred in both groups at the same pace. Meanwhile, the results of hypothesis (2) indicate that there was no mean difference between the treatment and control groups. As a result, the interaction effect of time and group interaction effect was also not significant, meaning that the pattern of brightness increase from week 0 to week 12 was similar for both groups. Therefore, the observed improvement over time cannot be attributed specifically to the Ascorbic Acid with ACTISOLV™ formulation but reflects a natural change that also occurred in the control group.

The insignificance of this interaction can be explained by two main factors. First, the improvement may be influenced by individual variability in natural skin responses, such as differences in hydration, barrier repair, and placebo-related effects, which vary across participants. These subject-specific responses may contribute to similar brightness increases in both groups. Second, as shown in Figure 3.2, the treatment group exhibited a highly heterogeneous response, with substantially greater variance than the control group. This large variance reduces the statistical power of the RM-ANOVA, making the mean increase shown in Figure 3.1 insufficient to reach statistical significance. So that, these factors explain why the pattern of brightness improvement over time did not differ between the treatment and control groups, resulting in a non-significant time \times group interaction effect.

(4) Tukey HSD Post Hoc Test

Following the significant results of the mean difference test across measurement times, a Tukey HSD test was conducted to identify which specific weeks showed significant differences in average skin brightness.

Table 3.4 Tukey HSD Post Hoc Results

Weeks (I)	Weeks (J)	Estimate	SE	p-value
0	2	-0.0498	0.0670	0.8789
	6	-0.1921	0.0626	0.0213
	12	-0.2202	0.0626	0.0068
2	6	-0.1423	0.0597	0.1001
	12	-0.1704	0.0759	0.1321
6	12	-0.0281	0.0521	0.9487

Based on the results of the Tukey HSD Based on the results of the Tukey HSD post hoc test presented in Table 3.4, two pairs of weeks exhibited statistically significant differences in mean skin brightness levels: between week 0 and week 6 ($p\text{-value} = 0.0213 < 0.05$), and between week 0 and week 12 ($p\text{-value} = 0.0068 < 0.05$). These findings indicate that the average skin brightness at weeks 6 and 12 differed significantly compared to week 0. Conversely, other week pairings did not demonstrate significant differences, as their p-values exceeded 0.05. Therefore, significant changes in skin brightness were primarily observed after weeks 6 and 12 relative to the baseline measurement.

JURNAL MATEMATIKA, STATISTIKA DAN KOMPUTASI
Dita Amelia, Suliyanto, Arinda Mahadesyawardani, Nur Azizah, Jovansha
Ariyawan, R. Arya Januarta Adiputra, Irsyad Yoga Adyatma

3. CONCLUSION

Based on the problem formulation and the results of this study, it can be concluded that there was a significant change in skin brightness over time, particularly at weeks 6 and 12 compared to week 0. However, no significant differences were found between the control group and the treatment group, nor was there a significant interaction between the observation time and the treatment group. This indicates that changes in skin brightness occurred naturally and similarly in both groups, without any specific influence from the use of creams containing or not containing the 20% Ascorbic Acid formula with ACTISOLV™.

REFERENCES

- [1] Angriyani, D. H., Dewi, N. K., & Setiawan, H., 2021. Pengaruh Model Pembelajaran TPS (Think Pair Share) Terhadap Keterampilan Berbicara Peserta Didik Kelas IV A SDN 5 Cakranegara Ajaran 2020/2021. *Renjana Pendidikan Dasar*, 1(3), 139.
- [2] Blanca, M. J., Arnau, J., García-Castro, F. J., Alarcón, R., & Bono, R., 2023. Non-normal Data in Repeated Measures ANOVA: Impact on Type I Error and Power. *Psicothema*, 35(1), 21–29. <https://doi.org/10.7334/psicothema2022.292>
- [3] Chilicka, K., Rogowska, A. M., Szyguła, R., Rusztowicz, M., & Nowicka, D., 2022. Efficacy of Oxybrasion in the Treatment of Acne Vulgaris: A Preliminary Report. *Journal of Clinical Medicine*, 11(13). <https://doi.org/10.3390/jcm11133824>
- [4] Danardono, 2014. *Analisis Data Longitudinal* (2nd ed.). Gajah Mada University Press.
- [5] Don, T.-M., Chen, M., & Huang, Y.-C., 2025. Incorporation of ascorbic acid-2-glucoside into ulvan microneedles to enhance its permeation for anti-aging and whitening treatment. *International Journal of Biological Macromolecules*, 292, 139250. <https://doi.org/https://doi.org/10.1016/j.ijbiomac.2024.139250>
- [6] Hassan, K., 2022. Applied Repeated Measures Design in medical field. *Journal of Kurdistan for Strategic Studies*. <https://doi.org/10.54809/jkss.vi11.19>
- [7] Heise, M. J., Mon, S. K., & Bowman, L. C., 2022. Utility of linear mixed effects models for event-related potential research with infants and children. *Developmental Cognitive Neuroscience*, 54, 101070. <https://doi.org/https://doi.org/10.1016/j.dcn.2022.101070>
- [8] Hildawati, Suhirman, L., Prisuna, B. F., Husnita, L., Mardikawati, B., Isnaini, S., Wakhyudin, Setiawan, H., Hadiyat, Y., Sroyer, A. M., & Saktisyahputra, 2024. *Buku Ajar Metodologi Penelitian Kuantitatif & Aplikasi Pengolahan Analisa* (1st ed.). PT. Sonpedia Publishing Indonesia.
- [9] Juarros-Basterretxea, J., Aonso-Diego, G., Postigo, Á., Montes-Álvarez, P., Menéndez-Aller, Á., & García-Cueto, E., 2024. Post-Hoc Tests in One-Way ANOVA: The Case for Normal Distribution. *Methodology*, 20(2 SE-Original Article). <https://doi.org/10.5964/meth.11721>
- [10] Langenberg, B., Helm, J. L., Günther, T., & Mayer, A., 2023. Understanding, Testing, and Relaxing Sphericity of Repeated Measures ANOVA with Manifest and Latent Variables Using SEM. *Methodology*, 19(1), 60–95. <https://doi.org/10.5964/meth.8415>
- [11] Montgomery, D. C., 2020. *Design and Analysis of Experiments* (10th ed. (ed.)). John Wiley & Sons, Inc.
- [12] Muhammad, L. N., 2023. Guidelines for repeated measures statistical analysis approaches with basic science research considerations. *Journal of Clinical Investigation*, 133(11), 11–14. <https://doi.org/10.1172/JCI171058>
- [13] Nanda, A., Mohapatra, D. B. B., Mahapatra, A. P. K., Mahapatra, A. P. K., & Mahapatra, A. P. K., 2021. Multiple comparison test by Tukey's honestly significant difference (HSD): Do

JURNAL MATEMATIKA, STATISTIKA DAN KOMPUTASI
Dita Amelia, Suliyanto, Arinda Mahadesyawardani, Nur Azizah, Jovansha
Ariyawan, R. Arya Januarta Adiputra, Irsyad Yoga Adyatma

- the confident level control type I error. *International Journal of Statistics and Applied Mathematics*, 6(1), 59–65. <https://doi.org/10.22271/maths.2021.v6.i1a.636>
- [14] Park, M., Won, K., Lee, J.-H., Park, Y., Oh, M., Chang, J., Lee, J., & Lee, M., 2025. Effects of Oral supplementation with eggshell membrane (Ovoderma®-AS) on skin health: A randomized, double-blind, placebo-controlled trial. *Journal of Functional Foods*, 127, 106713. <https://doi.org/https://doi.org/10.1016/j.jff.2025.106713>
- [15] Rachata Prokarug, Waranon Kongsong, & Chaleeporn Thammapornram, 2021. Analysis Ofemission Factor in Thailand for Vehicle Transportation That Affects the Carbon Footprint in the Production Process of Thai Dendrocalamus Giganteus Bamboo (Tdg)Laminated. [https://iaeme.Com/Home/Journal/IJARET63editor@iaeme.ComInternational Journal of Advanced Research in Engineering and Technology \(IJARET\)](https://iaeme.Com/Home/Journal/IJARET63editor@iaeme.ComInternational%20Journal%20of%20Advanced%20Research%20in%20Engineering%20and%20Technology%20(IJARET),%2012(11),%2063-74), 12(11), 63–74.
- [16] Sass, J., McCraw, T., & Paul Baek, J., 2023. *Increasing ascorbic acid performance*. Mendeley Data. <https://doi.org/10.17632/wb98x9mgmz.1>
- [17] Shrotri, S., Daamen, A., Kingsmore, K., Bachali, P., Grammer, A., & Lipsky, P., 2025. Transcriptomic Analysis Identifies Disease Severity and Therapeutic Response in Psoriasis. *JID Innovations: Skin Science from Molecules to Population Health*, 5(2), 100333. <https://doi.org/10.1016/j.xjidi.2024.100333>
- [18] Taris, T. W., Kessler, S. R., & Kelloway, E. K., 2021. Strategies addressing the limitations of cross-sectional designs in occupational health psychology: What they are good for (and what not). *Work & Stress*, 35(1), 1–5. <https://doi.org/10.1080/02678373.2021.1888561>
- [19] Zerbinati, N., Sommatis, S., Maccario, C., Di Francesco, S., Capillo, M. C., Rauso, R., Herrera, M., Bencini, P. L., Guida, S., & Mocchi, R., 2021. The Anti-Ageing and Whitening Potential of a Cosmetic Serum Containing 3-O-ethyl-l-ascorbic Acid. *Life (Basel, Switzerland)*, 11(5). <https://doi.org/10.3390/life11050406>
- [20] Zhou, Y., Zhu, Y., & Wong, W. K., 2023. Statistical tests for homogeneity of variance for clinical trials and recommendations. *Contemporary Clinical Trials Communications*, 33, 101119. <https://doi.org/https://doi.org/10.1016/j.conctc.2023.101119>