STUDY REPORT

SPLIT ENDS REDUCTION

REPORT: KAH012-24-D-1-R0, ISSUE DATE 04/25/2024

PROPOSAL: KAH012-24, REVISION 0 / KAH009-23, REVISION 9

INVESTIGATIONAL PRODUCTS

Hair Serum + 5% Cacay Oil
Hair Serum + 5% Argan Oil
Hair Serum Control

SPONSOR

KAHAI SAS
NIT: 900,314,941-9 - AK 15 # 134A-55 OF 702 - Colombia

CHRONOLOGY

SAMPLE RECEIPT DATE	*STUDY START*	*STUDY END*
03/18/2024	03/26/2024	04/19/2024

PERFORMED BY:
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Summary

STUDY REPORT

Split Ends Reduction

REPORT KAH012-24-D-1-R0; STUDY PLAN: KAH012-24-D-R0 / KAH009-23-D-R2

Study director

Nathana C. B. S. Ramos

INVESTIGATIONAL PRODUCTS

Official name	Sample ID
Hair Serum + 5% Cacay Oil	PI-KAH009-23-1
Hair Serum + 5% Argan Oil	PI-KAH009-23-3
Hair Serum Control	PI-KAH012-24-1

STUDY OBJECTIVE

Assess the potential of cosmetic prototypes to reduce split ends on hair tresses.

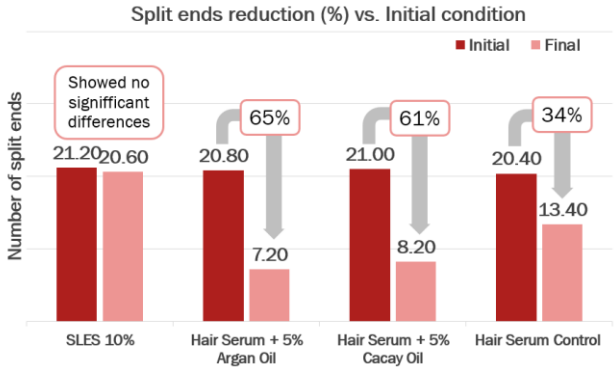
METHODOLOGICAL DESCRIPTION

Eighteen tresses of natural Caucasian hair (2.5g | 25cm, presenting at least 20 split-ends/100 hair fibers) had the number of split ends obtained (**Initial**). Then, the hair tresses were submitted to the treatments:

- SLES 10% (data from KAH009-23) – group **CTRL.**
- Hair Serum + 5% Cacay Oil (data from KAH009-23) – group **T01.**
- Hair Serum + 5% Argan Oil (data from KAH009-23) – group **T02.**
- Hair Serum Control – group **T03.**

After the products application and drying in a controlled environment (22 ± 2°C | 55 ± 5% R.H.) for 24 hours, and a new counting was performed on 100 hair fibers/ tresses (**Final**).

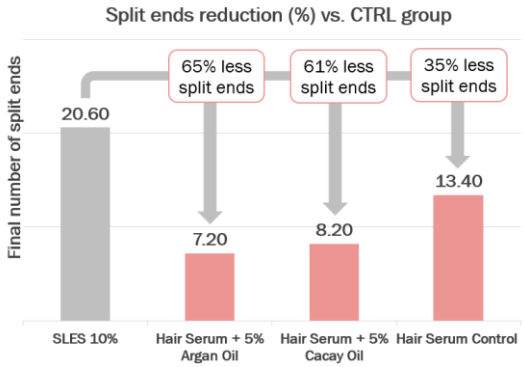
MAIN RESULTS



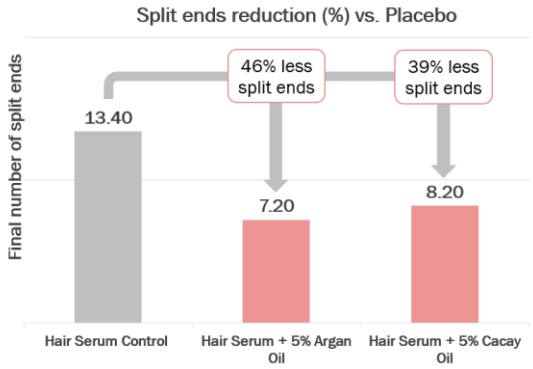
REDUCTION OF SPLIT ENDS, RS (% AND NUMBER OF TIMES) VS. INITIAL CONDITION

Treatment compared	Rs (%)	Rs (n)
CTRL	*	*
T01	61	3
T02	65	3
T03	34	2

**Showed no significant differences*



REDUCTION OF SPLIT ENDS, RS (% AND NUMBER OF TIMES) VS. CTRL GROUP.		
Treatment compared	Rs (%)	Rs (n)
T01	60	3
T02	65	3
T03	35	2



REDUCTION OF SPLIT ENDS, RS (% AND NUMBER OF TIMES) VS. T03 GROUP.		
Treatment compared	Rs (%)	Rs (n)
T01	39	2
T02	46	2

CONCLUSIONS

According the obtained results, the treatment **Hair Serum + 5% Cacay Oil** showed the required efficacy to support the following claims:

- ✓ Repair of split ends**.
- ✓ Reduction of split ends**.
- ✓ **60% (3 times)** fewer split ends**.
- ✓ **39% (2 times)** fewer split ends***.

The treatment **Hair Serum + 5% Argan Oil** showed the required efficacy to support the following claims:

- ✓ Repair of split ends**.
- ✓ Reduction of split ends**.
- ✓ **65% (3 times)** fewer split ends**.
- ✓ **46% (2 times)** fewer split ends***.

***When compared to shampoo without conditioning agent.*

****When compared to placebo group.*

Table of contents

1. Introduction

Measuring hair physical and chemical properties is an important procedure to develop new formulations, as these properties are closely related to the geometry and conservation status of hair fibbers.

The construction of the cortex is the main factor determining hair fibber mechanical properties. However, without the cuticle, this structure would be quickly changed. Cuticle is the external region of hair fibbers and is made of long, scale-like cells, arranged in overlapping layers and it may include 6 to 10 layers. The free ends of these cells are directed to the end of the fibber and, as they are attacked by the environment, these free ends get dried and loosen, causing the cortex to become unprotected.

Any elastic material, when it suffers the action from a tensioning force, will undergo deformation (strain) proportional to the force applied. Similarly, we say that for every strain, a stress is associated, which shows its tendency to recover back to its usual condition. The unit of stress is expressed as a force per unit area (F/A). The ratio of stress to strain is called the Young's modulus.

As hair fibbers suffer the action from a tensioning force, it develops a relation between the developed force by the hair fibber tensioned and its deformation. In the obtained curve, three main regions can be seen: the elastic region, the plastic region and the post-plastic region. In the elastic region (Hookean region), microfibrils have a β-keratin structure, while other regions microfibrils have a α-keratin structure.

The Hookean region of the curve, comprehended between approximately 0 to 2% elongation, is the one in which strain is proportional to the load applied. In this region, hair has a behavior that is similar to the one of a crystalline solid, having its shape structure homogeneously resistant to tension. This resistance is supplied especially due to hydrogen bonds, responsible for stabilizing the α-helix that keeps its structure of stretched amorphous chains. The ratio between tension and deformation taken in this region provides hair fibber its elastic modulus (Young's modulus).

In the region between approximately 2% and 25%-30% elongation, strain increases very rapidly without noticeable change in the stress applied. This change is explained by the conversion of microfibrils internal structure. When the hair fibber is not strained, microfibrils are formed essential by α-keratin, in which chains are arranged in compact patterns; in the plastic region a conversion takes place to the β-keratin structure, with chains being completely expanded, in a matrix of viscous liquid that does not offers any resistance to the tension applied. In this region, hair behaves like liquid or almost perfectly plastic material. This region is called the yield region.

In the post-yield region, which is reached when 30% deformation is exceeded, the strain and the stress become proportional again, being the region where breakage of the fibber occur. In it, the system again behaves as a crystalline solid. Resistance to deformation is due to the β-helix structure, and elongation occurs until the fibber breaks. The shape of the curve in this region depends mainly on cystine content in the fibber.

When hair is wet, the plastic zone begins at approximately half the tension required vs. dry hair, as a result from the loss of hydrogen bonds and coulombic interactions. Temperature increase has an effect that is similar to increasing fibber moisturization level; therefore, these two variables need to be carefully controlled when studies of this type are performed.

Hair break tests provides for the assessment of how hair behaves after different treatments when exposed to successive combability procedures. Using straightening lotions or perms, bleaching processes, cosmetic treatment, or thermal change procedures change the hair fibber characteristic morphology and these changes can increase the hair fibber fatigue process due to brushing process, causing hair fibbers to break.

2. Objective

Assess the potential of cosmetic prototypes to reduce split ends on hair tresses.

PROPOSED CLAIMS
Repair of split ends
Reduction of split ends
X% fewer split ends

3. Materials

3.1. Investigational Products

IDENTIFICATION	PRODUCT 1	PRODUCT 2	PRODUCT 3
Label ID	HAIR SERUM ACEITE DE CACAY	HAIR SERUM ACEITE DE ARGAN	SERUM CONTROL
Official name	Hair Serum + 5% Cacay Oil	Hair Serum + 5% Argan Oil	Hair Serum Control
Received quantity	1 flask	1 flask	1 flask
Date of receipt	03/18/2024	03/18/2024	03/18/2024
Sponsor code Batch Manufacturing Expiration	NIP B: 080324 Manuf.: 03/2024 Expire: 03/2025	NIP B: 080324-2 Manuf.: 03/2024 Expire: 03/2025	NIP B: 080324-1 Manuf.: 03/2024 Expire: 03/2025

NIP = Not informed | provided by the sponsor

Additional information about the investigational product(s) can be found in **Appendix 1**.

3.1.1. Application method

INVESTIGATIONAL PRODUCTS APPLICATION METHOD	
Product	Application method
SLES 10%	a) Wet the tress for 20 seconds and remove the excess of water. b) Apply 0.5 mL of product and rub for 60 seconds. Rinse the tress for 30 seconds and remove excess water.
HAIR SERUM	a) Wet the tress for 20 seconds and remove the excess of water. b) Apply 0.25 mL of product and rub for 60 seconds. Not to rinse.

3.1.2. Study coding

TREATMENT GROUPS	
Products	Study code
SLES 10%	CTRL
Hair Serum + 5% Cacay Oil	T01
Hair Serum + 5% Argan Oil	T02
Hair Serum Control	T03

3.1.3. Storage

Every Investigational Product/test item has been properly identified with a unique code in order to ensure traceability of the process according to procedure "TEC-005 - Control of Receipt of Investigational Product-Test Item".

After the end of the study, samples are properly disposed of through an incineration process carried out by a company duly approved by Kosmoscience®, according to procedure TEC-006 - Waste Management.

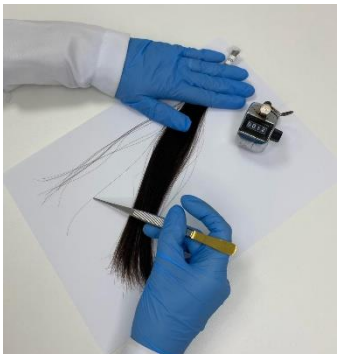
According to internal procedures, except by the request of the sponsor, to be made during the proposal of their project, the samples used during the study are discarded 6 months after the delivery of the final report.

3.2. Object of the study

Twenty tresses were prepared from natural Caucasian hair (2.5 g | 25 cm).

4. Methodology

4.1. Methodological description

Pre cleaning / Initial	Treatment	Final
		
• SLES 10% solution + 1min of massage + rinse for 30s. • 24 hours of drying (22±2°C| 55±5% R.H).	• **Initial counting:** at least 20 split-ends/100 hair fibers, visual/naked eye counting. Treatments application.	24 hours of drying (22±2°C| 55±5% R.H). • **Final counting**: 100 hair fibers/ tress, visual/naked eye counting.

4.1.1. Environmental conditions

All study was conducted in a controlled environment at 55 ± 5% relative humidity and 22 ± 2°C.

4.2. Variables and Data collection procedures

Data of number of Split Ends in 100 fibers collected from each tress.

A first counting was performed to evaluate the presence of a minimum number of split-ends (at least 20 split-ends/100 hair fibers Visual/Naked eye counting) (initial counting - **Initial**).

After the products application and drying in a controlled environment for 24 hours (22 ± 2 °C | 55 ± 5% R.H.). A new counting was performed on 100 hair fibers/ tresses (final counting - **Final**).

Illustrating images of hair tips for the treatment groups, obtained via digital pictures of last 5 cm of hair tresses.

4.3. Data analysis procedures

Statistical analysis

1) **Comparison of measurements (Initial and Final):** Initial vs. Final were statistically compared using bimodal, paired Student's t-test, considering a 95% confidence interval.

2) **Comparison with CTRL or T03 groups:** the number of split-ends were using one-way ANOVA, followed by a Dunnet's post-test, considering a 95% confidence interval.

3) **Comparison between treatments:** the number of split-ends were compared to each other using bimodal, unpaired Student's t-test, considering a 95% confidence interval.

The complete data of statistical analysis are described on **Appendix 3.**

Calculations:

Using the **Equations 1** and **2** the results of "Reduction of Split Ends, ***Rs***", were obtained, in percentage and in number of times, respectively, calculated for treatment group compared to **CTRL** or **T03** groups.

$$Rs = 100 * \left(\frac{S_{CTRL} - S_{TRT}}{S_{CTRL}}\right)$$

Equation 1. Calculation of Reduction in Split Ends (%) of the treatments compared to Condition 01. Where S = number of split ends.

$$Rs = \frac{S_{CTRL}}{S_{TRT}}$$

Equation 2. Calculation of Reduction in Split Ends (number of times) of the treatments compared to Condition 01. Where S = number of split ends.

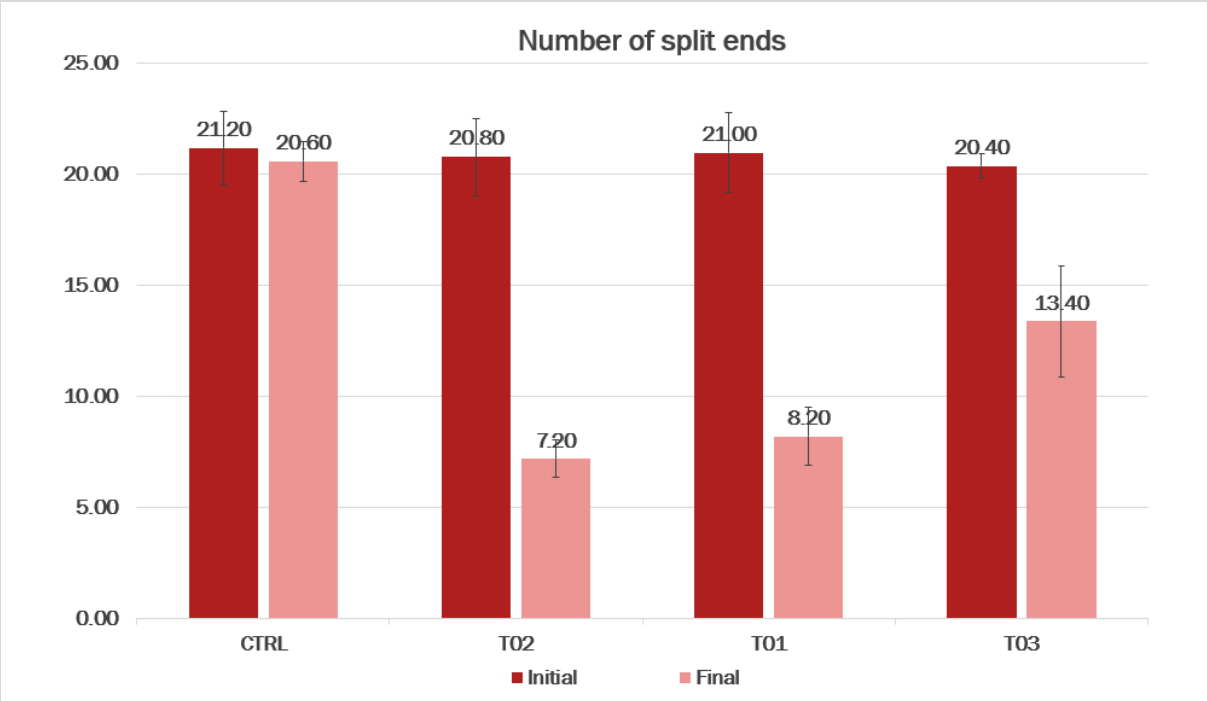
4.3.1. Computer programs used

GraphPad™ Prism® 8.4.3.

5. Results and discussions

The study was conducted according to the study plan KAH012-24-D-R0.

Graph 1 illustrates the data obtained in terms of relative number of Split Ends in 100 fibers collected from each tress.



Graph 1. Number of split ends. Mean ± standard deviation.

Table 1 shows the results of statistical analysis and Reduction of split-ends, ***Rs***, in percentage and number of times.

TABLE 1. STATISTICAL ANALYSIS. C.I. 95%. REDUCTION OF SPLIT ENDS (% AND NUMBER OF TIMES).

Comparison	Showed significant differences?	Rs (%)	Rs (n° of times)
CTRL Initial vs. CTRL Final	No	N/A	N/A
T01 Initial vs. T01 Final	Yes	61	3
T02 Initial vs. T02 Final	Yes	65	3
T03 Initial vs. T03 Final	Yes	34	2
CTRL vs. T01	Yes	60	3
CTRL vs. T02	Yes	65	3
CTRL vs. T03	Yes	35	2
T03 vs. T01	Yes	39	2

TABLE 1. STATISTICAL ANALYSIS. C.I. 95%. REDUCTION OF SPLIT ENDS (% AND NUMBER OF TIMES).			
Comparison	Showed significant differences?	Rs (%)	Rs (n° of times)
T03 vs. T02	Yes	46	2
T01 vs. T02	No	N/A	N/A

N/A: no applicable.

The tresses submitted to the treatment:

- ✓ **CTRL** showed no significantly difference in number of split ends when compared to **Initial** condition (tresses without treatment).
- ✓ **T01, T02** and **T03** showed significantly lower number of split ends when compared to respective **Initial** condition (tresses without treatment) and when compared to **CTRL** group.
- ✓ **T01** and **T02** showed significantly lower number of split ends when compared to **T03** group.
- ✓ **T01** and **T02** showed no significantly difference in number of split ends when compared to each other.

6. Conclusions

According the obtained results, the treatment **Hair Serum + 5% Cacay Oil** showed the required efficacy to support the following claims:

- ✓ Repair of split ends**.
- ✓ Reduction of split ends**.
- ✓ **60% (3 times)** fewer split ends**.
- ✓ **39% (2 times)** fewer split ends***.

The treatment **Hair Serum + 5% Argan Oil** showed the required efficacy to support the following claims:

- ✓ Repair of split ends**.
- ✓ Reduction of split ends**.
- ✓ **65% (3 times)** fewer split ends**.
- ✓ **46% (2 times)** fewer split ends***.

***When compared to shampoo without conditioning agent.*
****When compared to placebo group.*

OBJECTIVES ACHIEVEMENT	
Intended objectives	Achieved objectives
Repair of split ends	✓
Reduction of split ends	✓
X% fewer split ends	✓

ADMINISTRATIVE HEAD:	TECHNICAL HEAD:	QUALITY HEAD:
Adriano S. Pinheiro	Nathana C. B. S. Ramos	Jefferson R. Bongarthner
Chemist CRQ-IV 04245232	Chemist CRQ-IV 04164749	Chemist CRQ-IV 04261924

7. Reports revision history

REVISION NUMBER	ISSUE DATE	DESCRIPTION, JUSTIFICATION OF REVISIONS
0	04/25/2024	Initial emission

8. Final ethical considerations

8.1. Quality assurance

The quality of the data is ensured through Kosmoscience®'s Integrated Management System, which guarantees data traceability, employee training, equipment calibration, and the use of standardized and/or validated methodologies.

The Quality Assurance Department performs audits of the Management System and is available to receive our clients for specific monitoring of their research.

The representative signature of the Quality Assurance System means that the research was conducted as described above.

8.2. Confidentiality

Kosmoscience® ensures the confidentiality of information through its compliance processes with sponsors as well as through its internal policies and procedures.

All employees are properly trained on the Code of Conduct (CC-001), LGPD Policy (LGPD-001), and Confidentiality and Security Procedure (RH-014).

8.3. Data recording and archiving

The results and interpretations described in this report refer only to the items tested and duly cited in this report.

All data was processed and stored according to procedure SQ-002 - Documents Issuance, Change and Approval; SQ-005 - Records Storage and Retrieval; electronic records according to procedure INF-001 - Management of Computer Resources; and presented according to procedure TEC-010 - Results Presentation; and TEC-007 - Study Report Preparation and Issuance Guide.

The electronic data was stored in folders and properly protected by password and login, being stored indefinitely.

The physical documentation was stored in properly protected files and under the custody of the archivist for 5 years, being destroyed after this period.

8.4. Copyright

9. Bibliographic references

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10. Appendix

APPENDIX 1: INVESTIGATIONAL PRODUCT(S) INFORMATION

The information below is entirely the Sponsor's responsibility:

TESTED PRODUCTS FORMULAS

Ingredients	Hair Serum Control	Hair Serum + 5% Argan Oil	Hair Serum + 5% Cacay Oil
INCI NAME	**%**	**%**	**%**
Water (Aqua)	87.4	82.4	82.4
Caryodendron orinocense seed oil (Cacay Oil)			5.0
Argania Spinosa Kernel Oil (Argan Oil)		5.0	
Propylene Glycol	4.0	4.0	4.0
Stearyl Alcohol	3.0	3.0	3.0
Behentrimonium Methosulfate (and) Quaternium-87 (and) Cetearyl Alcohol	3.0	3.0	3.0
Cetyl Alcohol	1.0	1.0	1.0
Phenoxyethanol (and) Benzyl Alcohol (and) Ethylhexylglycerin (and) Tocopherol	1.0	1.0	1.0
Tocopheryl Acetate	0.5	0.5	0.5
Disodium EDTA	0.1	0.1	0.1
TOTAL	**100.0**	**100.0**	**100.0**

APPENDIX 2: OBTAINED DATA

NUMBER OF SPLIT ENDS								
n	Initial				Final			
	CTRL	T01	T02	T03	CTRL	T01	T02	T03
1	20	20	20	21	20	8	6	14
2	24	24	20	21	22	9	7	14
3	20	21	20	20	20	9	8	17
4	21	20	24	20	20	7	9	11
5	21	20	20	20	21	8	6	11
Mean	21.20	21.00	20.80	20.40	20.60	8.20	7.20	13.40
SD	1.64	1.73	1.79	0.55	0.89	0.84	1.30	2.51

APPENDIX 3: STATISTICAL ANALYSIS

Method: bimodal, paired Student's T-test. Confidence interval: 95%.

Data: Number of split ends, listed on **Appendix 2**. ***Initial vs. Final.***

Software: GraphPad™ Prism® 8.4.3

Paired t test	CTRL	T01
P value	0.208	<0,0001
P value summary	ns	****
Significantly different (P < 0.05)?	No	Yes
One- or two-tailed P value?	Two-tailed	Two-tailed
t, df	t=1,500, df=4	t=21,95, df=4
Number of pairs	5	5
How big is the difference?		
Mean of differences (C - A)	-0.6	-12.8
SD of differences	0.8944	1.304
SEM of differences	0.4	0.5831
95% confidence interval	-1,711 to 0,5106	-14,42 to -11,18
R squared (partial eta squared)	0.36	0.9918
How effective was the pairing?		
Correlation coefficient (r)	0.9186	0.6901
P value (one tailed)	0.0138	0.0986
P value summary	*	ns
Was the pairing significantly effective?	Yes	No

Paired t test	T02	T03
P value	<0,0001	0.0031
P value summary	****	**
Significantly different (P < 0.05)?	Yes	Yes
One- or two-tailed P value?	Two-tailed	Two-tailed
t, df	t=26,67, df=4	t=6.390, df=4
Number of pairs	5	5
How big is the difference?		
Mean of differences (C - A)	-13.6	-7.000
SD of differences	1.14	2.449
SEM of differences	0.5099	1.095
95% confidence interval	-15,02 to -12,18	-10.04 to -3.959
R squared (partial eta squared)	0.9944	0.9108
How effective was the pairing?		
Correlation coefficient (r)	0.7717	0.2182
P value (one tailed)	0.0632	0.3622
P value summary	ns	ns
Was the pairing significantly effective?	No	No

Method: One-way ANOVA with Dunnett's post-test. Confidence interval: 95%.

Data: Number of split ends, listed on **Appendix 2**. ***CTRL vs. Treatments***

Software: GraphPad™ Prism® 8.4.3

ANOVA summary			
F	79.24		
P value	<0.0001		
P value summary	****		
Significant diff. among means (P < 0.05)?	Yes		
R squared	0.9369		
ANOVA table	SS	DF	MS
Treatment (between columns)	564.6	3	188.2
Residual (within columns)	38.00	16	2.375
Total	602.6	19	
Dunnett's multiple comparisons test	Mean Diff,	95,00% CI of diff,	Significant?
CTRL vs. T01	12.40	9.873 to 14.93	Yes
CTRL vs. T02	13.40	10.87 to 15.93	Yes
CTRL vs. T03	7.200	4.673 to 9.727	Yes

Method: One-way ANOVA with Dunnett's post-test. Confidence interval: 95%.

Data: Number of split ends, listed on **Appendix 2**. *T03 vs. Treatments*

Software: GraphPad™ Prism® 8.4.3

ANOVA summary			
F	19.1		
P value	0.0002		
P value summary	***		
Significant diff. among means (P < 0.05)?	Yes		
R squared	0.7610		
ANOVA table	SS	DF	MS
Treatment (between columns)	110.8	2	55.40
Residual (within columns)	34.80	12	2.900
Total	145.6	14	
Dunnett's multiple comparisons test	Mean Diff.	95.00% CI of diff.	Significant?
T03 vs. T01	5.200	2.505 to 7.895	Yes
T03 vs. T02	6.200	3.505 to 8.895	Yes

Method: bimodal, unpaired Student's T-test. Confidence interval: 95%.

Data: Number of split ends, listed on **Appendix 2**. *T01 vs. T02*

Software: GraphPad™ Prism® 8.4.3

Unpaired t test	
P value	0.1869
P value summary	ns
Significantly different (P < 0.05)?	No
One- or two-tailed P value?	Two-tailed
t, df	t=1,443, df=8
How big is the difference?	
Mean of column E	8.2
Mean of column F	7.2
Difference between means (F - E) ± SEM	-1,000 ± 0,6928
95% confidence interval	-2,598 to 0,5976
R squared (eta squared)	0.2066
F test to compare variances	
F, DFn, Dfd	2,429, 4, 4
P value	0.4112
P value summary	ns
Significantly different (P < 0.05)?	No

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