Grupo
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STUDY REPORT

STUDY ON THERMAL PROTECTION OF HAIR

REPORT: KAH009-23-D-3-R1, ISSUE DATE 04/02/2024

PROPOSAL: KAH009-23, REVISION 9

INVESTIGATIONAL PRODUCT

HAIR SERUM CONTROL
Hair Serum + 5% Cacay Oil
Hair Serum + 5% Argan Oil

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	03/27/2024	03/28/2024

PERFORMED BY:
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Summary

STUDY REPORT

Study on Thermal Protection of Hair

REPORT KAH009-23-D-3-R1; STUDY PLAN: KAH009-23-D-R2

Study director

Nathana C. B. S. Ramos

INVESTIGATIONAL PRODUCT

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-KAH009-23-4

STUDY OBJECTIVES

Assessing the potential of cosmetic prototypes to protect the α-keratin structure of the thermally damaged hair via the DSC (Differential Scanning Calorimetry) technique.

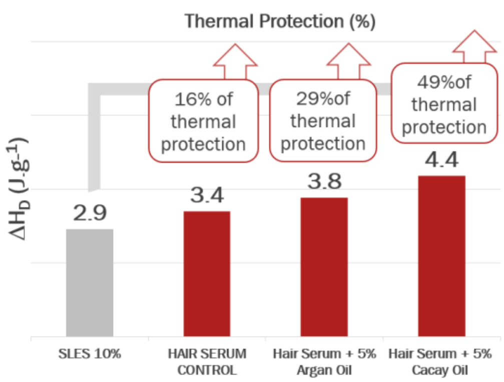
METHODOLOGICAL DESCRIPTION

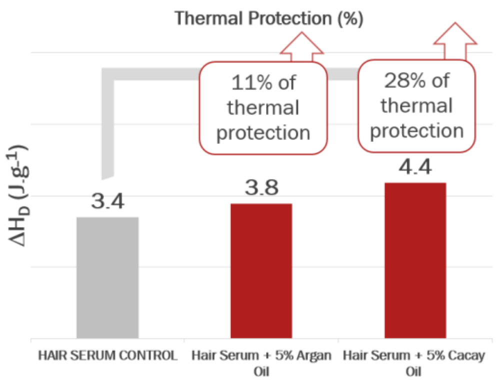
Tresses of Natural Caucasian hair were submitted to the treatments:

- SLES 10% – group **CTRL.**
- HAIR SERUM CONTROL – group **T01**
- Hair Serum + 5% Cacay Oil – group **T02.**
- Hair Serum + 5% Argan Oil – group **T03.**

After the treatments, the tresses were submitted to two different thermal damage procedure (Dryer 80 °C and + straightening iron 230 °C). Hair fibers were randomly collected for DSC analysis using the equipment Hitachi High-Tech Sciences TA7000 Series Simultaneous Thermogravimetric Analyzer (30 °C to 300 °C).

MAIN RESULTS





CONCLUSIONS

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

- **49%** of thermal protection.
- Thermal protection at 230°C.

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

- **29%** of thermal protection.
- Thermal protection at 230°C.

*****_When compared to the hair treated with shampoo without conditioning agent._

Table of contents

1. Introduction

The industry of personal care, cosmetics and perfumery tries to stimulate consumers to acquire products via increasingly effective marketing strategies. The purpose of such strategies is to provoke the consumers' mind via use of words, images and concepts, and create a psychological need to try the product. However, the need exists to support the product claims scientifically, both to justify them before increasingly demanding consumers and to ensure success and prosperity of the brand that must keep its marketing promises.

In this context, we try to make a practical and effective application of scientific study techniques that, until then, were primarily intended to academic purposes.

The DSC technique was used by Cao[2] to observe the thermal behavior of hair. Wortmann3 assessed the denaturation of keratin in hair fibers by means of DSC. This technique measures the thermal energy supplied to a material to make physical changes such as vaporization, fusion and phase transition of materials. In his study, Cao[2] found two characteristic bands, one in the 0 – 200°C range related to water in hair and the other between 250 and 280°C, related to phase transition of the crystalline phase α-keratin.

Belletti *et al.*[4] researched the DSC technique linked to gas chromatography to measure the amount of water in hair fibers. Ehen *et a*l.[5] studied the decomposition of four types of fibers using a combination of DSC and TG techniques.

The thermal analysis of keratin in water by differential thermal analysis (DTA) or differential scanning calorimetry (DSC) are techniques suitable to determine the binding strength of water molecules via the energy required to remove such molecules[4]. Changes in α-helicoid structures of intermediate filaments and the physical / chemical effects from cosmetic treatments can also be investigated[1,3].

2. Objectives

Assessing the potential of cosmetic prototypes to protect the α-keratin structure of the thermally damaged hair via the DSC (Differential Scanning Calorimetry) technique.

PROPOSED CLAIMS
X% (or X times) of thermal protection
Thermal protection at 230°C

3. Materials

3.1. Investigational Product

IDENTIFICATION	PRODUCT 1	PRODUCT 2	PRODUCT 3
Label ID	HAIR SERUM ACEITE DE CACAY	HAIR SERUM ACEITE DE ARGAN	HAIR 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 PRODUCT APPLICATION METHOD	
Product	Mode of use
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 the excess of 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 CONTROL	T01
Hair Serum + 5% Cacay Oil	T02
Hair Serum + 5% Argan Oil	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 study

Twelve tresses were prepared from Natural Caucasian hair (2.5g | 25cm long each).

4. Methodology

4.1. Methodological description

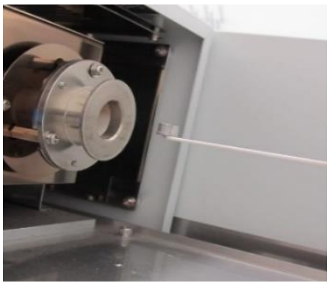
Pre Cleaning | Treatment

- **Pre cleaning:** 10% SLES solution + rub for 1 minute + rinse for 30 seconds.
- Treatments application.

Termic Damage

- Blow air dryer 10min - automatic combing machine (80°C | 25rpm).
- Straightening iron 10 strokes (10s each) at 230°C.

Measurements



- DSC analyzes: 3 sets of data on thermal protection per treatment.
- Equipment High-Tech Sciences TA7000.

4.1.1. Environmental conditions

All the experiment was performed in a standardized environment at 22 ± 2 °C and 55 ± 5% of relative humidity.

4.2. Variables and Data collection procedures

The DSC data showed details of the endothermic peak relating to ΔH and T of α-keratin denaturation, enthalpy and temperature, respectively. The complete data for ΔH_D and T_D obtained for the study groups is shown on **Appendix 2.**

The device used was the Hitachi High-Tech Sciences TA7000 Series Simultaneous Thermogravimetric Analyzer, STA7200, **Figure 1.** This instrument has been designed to simultaneously perform Thermogravimetric (TG) and Differential Scanning Calorimetric (DSC) measurements.

The analyses were made in triplicate with approximately 10 mg of chopped hair. The flow used was 100 mL/min of nitrogen gas with heating rate of 10°C/min. The analyses were made from 30°C to 300°C.

Figure 1. Hitachi High-Tech Sciences TA7000 Series Simultaneous Thermogravimetric Analyzer, STA7200 equipment.

4.3. Data analysis procedures

Statistical analysis:

1) **Comparison with CTRL group:** one-way ANOVA, followed by a Dunnett's post-test, considering a 95% confidence interval.

2) **Comparison between treatments:** one-way ANOVA, followed by a Tukey's post-test, considering a 95% confidence interval.

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

Calculations:

Using **Equation 1** the results of **"Percentage of Thermal Protection, P_{TP}%"** were obtained, calculated for treatment group compared to **CTRL** and **Control serum** groups.

$$P_{PT}\% = 100 * \left(\frac{\Delta H_{D\,TRT} - \Delta H_{D\,CTRL}}{\Delta H_{D\,CTRL}}\right)$$

Equation 1. Percentage of Thermal Protection, P_{TP} (%) of study groups compared to CTRL and Control serum group.

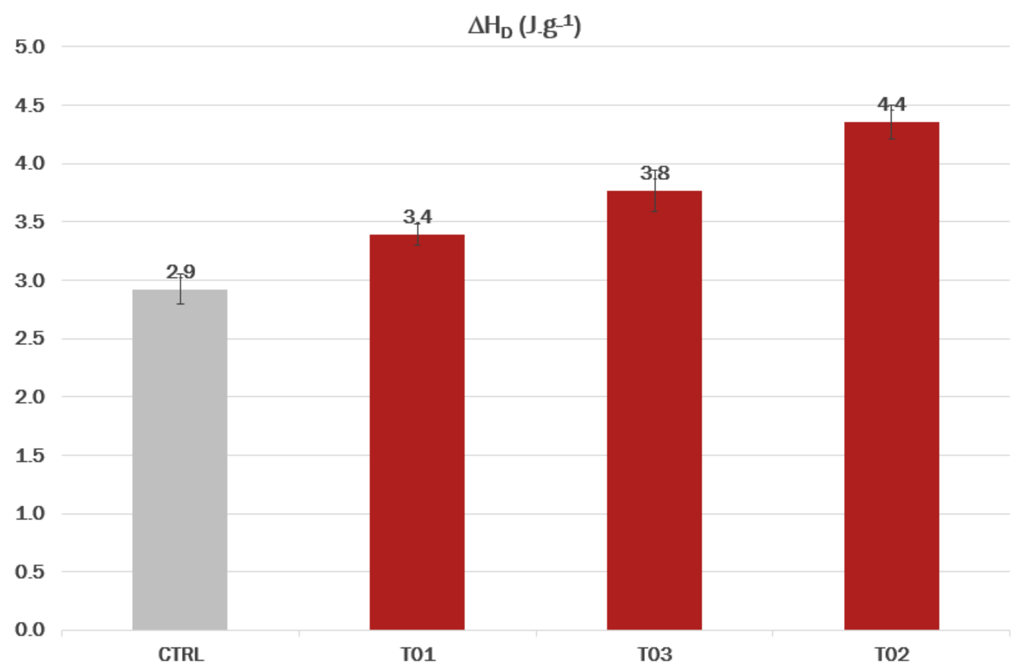
4.3.1. Computer programs used

GraphPad™ Prism® 8.4.3.

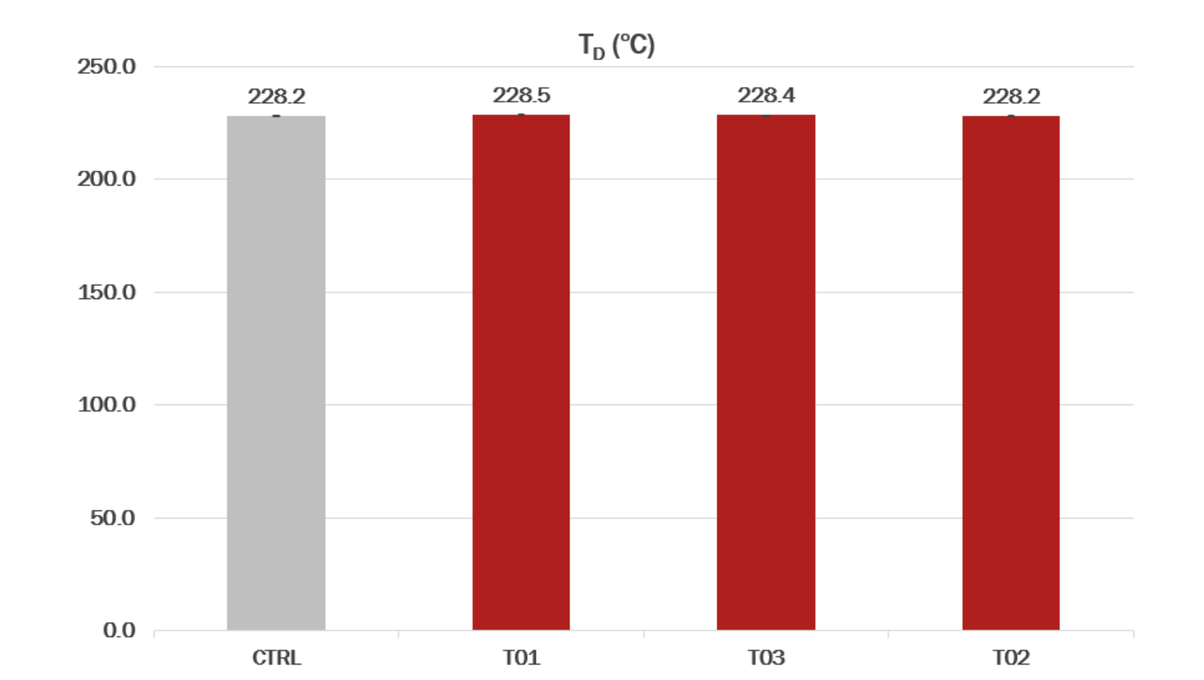
5. Results and discussions

The study was conducted according to the study plan KAH009-23-D-R2.

Graphs 1 and **2** illustrates the mean values of denaturation enthalpies and temperatures of α-keratin of the analyses performed in the temperature range between 30 °C and 300 °C.



Graph 1. ΔH values of α-keratin denaturation of the study groups. Mean ± standard deviation. N = 3.



Graph 2. T values of α-keratin denaturation of the study groups. Mean ± standard deviation. N = 3.

Table 1 shows the results of statistical analyses, and the results of **Potential of Thermal Protection,** in percentage, promoted by the treatment.

TABLE 1. STATISTICAL ANALYSIS. C.I. 95%. POTENTIAL OF THERMAL PROTECTION, P_{PT} – CTRL VS. TRT

Comparison	Showed significant differences?		$\%P_{PT}$
	ΔH_D	T_D	
CTRL vs. T01	Yes	No	**16**
CTRL vs. T02	Yes	No	**49**
CTRL vs. T03	Yes	No	**29**
T01 vs. T02	Yes	No	**28**
T01 vs. T03	Yes	No	**11**
T02 vs. T03	Yes	No	N/A

N/A – not applicable

The tresses submitted to the treatment:

- ✓ **T01, T02** and **T03** showed **ΔH_D** values significantly higher compared to the **CTRL** group.
- ✓ **CTRL, T01, T02** and **T03** showed no significant differences of T_D values when compared to each other.
- ✓ **T02** and **T03** showed **ΔH_D** values significantly higher compared to the **T01** group.
- ✓ **T02** showed **ΔH_D** values significantly higher compared to **T03** group.

6. Conclusions

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

- **49%** of thermal protection.
- Thermal protection at 230°C.

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

- **29%** of thermal protection.
- Thermal protection at 230°C.

**When compared to the hair treated with shampoo without conditioning agent.*

OBJECTIVES ACHIEVEMENT	
Proposed Claims	Achieved claims
X% (or X times) of thermal protection	✓
Thermal protection at 230°C	✓

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

7. Reports revision history

REVISION NUMBER	ISSUE DATE	DESCRIPTION, JUSTIFICATION OF REVISIONS
0	04/02/2024	Initial emission
1	04/02/2024	Inclusion of products official name. Change of products position on graphics. Inclusion of product's formula

We declare that the previous revision 0 is obsolete as of the date of this current revision number 1. Any reproduction of data from revision 0 (previously issued) becomes impracticable and invalid from this new revision 1

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

This document is authorized for internal use by the sponsor, as well as by government regulatory agencies, for the evaluated product.

The sponsor of the study may cite the original document and use the findings as a reference in materials for their own disclosure.

9. Bibliographic references

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2. CAO. J. Melting study of the α-form crystallites in human hair keratin by DSC. Termodinamic Acta, p. 335, 1999.
3. WORTMANN, F. J.; SPRINGOB, C.; SENDELBACH, G. Investigations of cosmetically treated human hair by differential scanning calorimetry in water. IFFCC 12, 2000.

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6. DIAS, T. C. S; DONOLATO, A.; GOMES, A. Hidratação e proteção dos cabelos e os silicones. Anais do Congresso ABC, São Paulo, 2000.

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

n	ΔH_D (J.g^{-1})			
	CTRL	T01	T02	T03
1	3.0715	3.2874	4.4275	3.9705
2	2.8724	3.4500	4.1905	3.6475
3	2.8278	3.4389	4.4486	3.6876
Mean	**2.9239**	**3.3921**	**4.3555**	**3.7686**
sd	**0.1297**	**0.0908**	**0.1433**	**0.1761**

n	T_D (°C)			
	CTRL	T01	T02	T03
1	228.2324	228.2600	228.3158	228.3508
2	228.1189	228.6929	228.1566	228.4940
3	228.2253	228.4798	228.2343	228.4298
Mean	**228.1922**	**228.4776**	**228.2356**	**228.4249**
sd	**0.0636**	**0.2165**	**0.0796**	**0.0717**

APPENDIX 3: STATISTICAL ANALYSIS

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

Data: ΔH_D and T_D values listed on **Appendix 2.** ***CTRL vs. treatments***

Software: GraphPad™ Prism® 8.4.3

One-way analysis of variance	ΔH_D		
F	57,38		
P value	<0,0001		
P value summary	****		
Significant diff. among means (P < 0.05)?	Yes		
R squared	0,9556		
ANOVA table	SS	DF	MS
Treatment (between columns)	3,297	3	1,099
Residual (within columns)	0,1532	8	0,01916
Total	3,451	11	
Dunnett's multiple comparisons test	Mean Diff,	95,00% CI of diff,	Significant?
CTRL vs. T01	-0,4682	-0,7936 to -0,1428	Yes
CTRL vs. T02	-1,432	-1,757 to -1,106	Yes
CTRL vs. T03	-0,8446	-1,170 to -0,5192	Yes

One-way analysis of variance	T_D		
F	3,761		
P value	0,0595		
P value summary	ns		
Significant diff. among means (P < 0.05)?	No		
R squared	0,5851		
ANOVA table	SS	DF	MS
Treatment (between columns)	0,176	3	0,05866
Residual (within columns)	0,1248	8	0,01559
Total	0,3007	11	
Dunnett's multiple comparisons test	Mean Diff,	95,00% CI of diff,	Significant?
CTRL vs. T01	-0,2854	-0,5790 to 0,008260	No
CTRL vs. T02	-0,04337	-0,3370 to 0,2503	No
CTRL vs. T03	-0,2327	-0,5263 to 0,06096	No

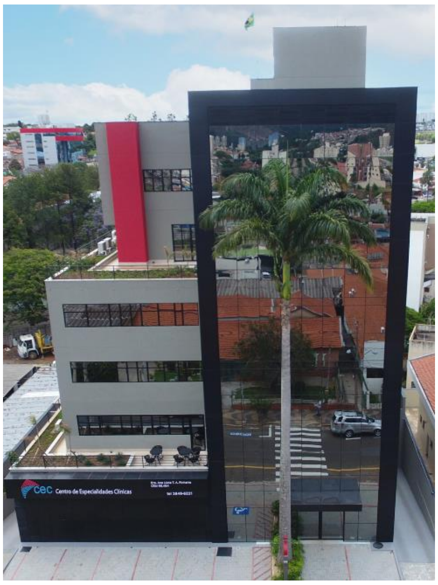
Method: One-way ANOVA with Tukey's post-test. Confidence Interval: 95%.

Data: ΔH_D and T_D values listed on **Appendix 2.** ***Comparison between treatments***

Software: GraphPad™ Prism® 8.4.3

One-way analysis of variance	ΔH_D		
F	35,49		
P value	0,0005		
P value summary	***		
Significant diff. among means (P < 0.05)?	Yes		
R squared	0,9221		
ANOVA table	SS	DF	MS
Treatment (between columns)	1,414	2	0,7072
Residual (within columns)	0,1196	6	0,01993
Total	1,534	8	
Tukey's multiple comparisons test	Mean Diff,	95,00% CI of diff,	Significant?
T01 vs. T02	-0,9634	-1,317 to -0,6098	Yes
T01 vs. T03	-0,3764	-0,7301 to -0,02277	Yes
T02 vs. T03	0,587	0,2333 to 0,9407	Yes

One-way analysis of variance	T_D		
F	2,499		
P value	0,1624		
P value summary	ns		
Significant diff. among means (P < 0.05)?	No		
R squared	0,4544		
ANOVA table	SS	DF	MS
Treatment (between columns)	0,09718	2	0,04859
Residual (within columns)	0,1167	6	0,01945
Total	0,2138	8	
Tukey's multiple comparisons test	Mean Diff,	95,00% CI of diff,	Significant?
T01 vs. T02	0,242	-0,1073 to 0,5913	No
T01 vs. T03	0,0527	-0,2966 to 0,4020	No
T02 vs. T03	-0,1893	-0,5386 to 0,1600	No



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