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Development of a sunscreen Product containing Epigallocatechin Gallate

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Development of an environmentally friendly sunscreen using epigallocatechin gallate

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Abbreviations

8-OHdG ..... *8-hydroxy-2' -deoxyguanosine*
ATRA ..... *all-trans-Retinoic acid,*
BHT ..... *Butylhydroxytoluol*
BSA ..... *Body surface area*
CD ..... *Cluster of differentiation*
CPD ..... *Cyclobutane pyrimidine dimers*
DNA ..... *Deoxyribonucleic Acid*
EGCG ..... *Epigallocatechin gallate*
EHMC ..... *Ethylhexyl methoxycinnamate*
ERK ..... *extracellular-signal regulated kinases*
GSH ..... *Glutathione*
HA ..... *Hyaluronic acid*
HEPES ..... *4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid*
HL ..... *Half-life*
HPV ..... *Human papillomavirus*
IBM ..... *International Business Machines*
JNK ..... *c-Jun N-terminal kinase*
LPO ..... *Lipid peroxidation*

MAPK.....*Mitogen-activated protein kinase*
MED .....*Minimal erythema dose*
N2O2 .....*Dinitrogen dioxide*
NOS ..... *Nitric oxide synthase*
O2 .....*Oxygen*
RSOM .....*Raster Scan Optoacoustic Mesoscopy*
RT .....*Room temperature*
SCH.....*Stratum corneum hydration*
SCORAD .....*Scoring atopic dermatitis*
SPF .....*Sun protecting factor*
SPSS.....*Statistical Package for the Social Sciences*
TEWL .....*Transepidermal water loss*
TiO_2 .....*Titanium dioxide*
UV ..... *Ultraviolet*
VEGF.....*Vascular endothelial growth factor*
ZIM.....*Das zentrale Innovationsprogramm Mittelstand*
ZiO.....*Zinc oxide*

Introduction

Epigallocatechin gallate (EGCG) is the most abundant polyphenol in green tea leaves, *Camellia sinensis.* The molecule is known for its anti-inflammatory and antioxidant properties, which have made green tea leaves a commonly used herbal therapy for hundreds of years. (Zink & Traidl-Hoffmann, 2015b)

EGCG has been reported to have protective effects on UV-induced skin damage. Multiple mechanisms have been described to play a role in protection against UV-damage:

- Direct UV-damage protection by inhibiting the activation process of MAPK and the phosphorylation process of ERK1/2, JNK and p38
- Antioxidant effects by inhibiting the production of free radicals, LPO, N2O2, NOS and the destruction of antioxidant enzymes
- Anti-inflammatory effect by preventing the destruction of Langerhans cells and macrophage and neutrophile migration
- Anticarcinogenic effect by preventing DNA damage and 8-OHdG production (Camouse et al., 2009; Katiyar, Afaq, et al., 2001; OyetakinWhite et al., 2012; Vayalil et al., 2003; Yang et al., 2009)

Based on the existing literature, it was the aim of the study to develop a sunscreen product on EGCG basis, which advantageously used the molecule properties for UV-damage prevention. The project was started by examining the scientific literature and reviewing the molecule stability, its interaction with common cosmetic ingredients and its therapeutic effect in the treatment of common dermatological conditions.

EGCG

Stability

The purpose of this literature research was to define the stability of the molecule of EGCG under different thermic, medium, pH conditions and to establish how different concentrations might affect the degradation process.

Concentration

Based on the existing literature it has been reported how better stability was achieved at higher concentrations. (Krupkova et al., 2016)
Sang et al. 2005 analyzed EGCG-concentration in water at body temperature (37°C). The 96 µM solution showed 5 times better stability than the 20 µM one after half an hour.
Furthermore, EGCG solutions of 0,51, 2,06 and 6,99 mM were stored at room temperature for a week. The two lower concentrated solutions achieved stability of circa 80%, whereas the 6,99 mM solution reached close to 100% stability. (Krupkova et al., 2016; Sang et al., 2005)
Different EGCG solutions (in HEPES buffer) in concentrations ranging from 0,0006 to 1,9651 mM showed a similar concentration-dependent stability, the higher concentrated solutions degraded less than the lower concentrated ones. (Fangueiro et al., 2014; Krupkova et al., 2016)
A 2012 study showed once again similar results. EGCG solution of 1,7 mg/ml and 5 mg/ml were stored at 80 degrees Celsius for 6h. They degraded to respectively ca. 50% and 85% of their previous concentrations. (Li et al., 2012) (Table I)
Concentrations of 0,1, 0,5 and 2 mg/ml were left under sun and air exposure for 2 days. The first two concentrations showed a complete degradation while the third one had lost less than 20%. (Zeng et al., 2018)

Temperature

EGCG solutions (in HEPES) were examined after being left for 48h at the respective temperatures of 4 and 25 degrees Celsius. The solutions stored at 4°C showed better stability than the ones at 25°C. For the 100 µg/mL solution in particular, the scientists reported 90% stability at the lower temperature compared to only 20% at RT. (Fangueiro et al., 2014) Li et al. 2012 analyzed the stability of green tea extract at temperatures ranging from 25 to 120 degrees Celsius. As previously speculated, the concentrates were less stable at higher temperatures. At 25°C it took almost 20 days to reach 90% degradation, at 120°C it took less than 4 minutes. (Li et al., 2012)

Thermic imbalance seems to not only influence the stability, but also to have an effect on the different degradation processes. If the temperatures are lower than 44°C the degradation process seems to be mainly oxidation, with raising temperatures epimerization seems to play a constantly growing role and after 99°C it becomes the main degradation process. (Wang et al., 2008)

These thermic degradation differences show a similar pattern as the interaction of temperature and pH values during the degradation process. pH-values seem to be the main cause of EGCG-degradation until a temperature of 25 degrees, after which the temperature itself seems to play an increasingly bigger role. (Proniuk et al., 2002)

Since most cosmetic and dermatologic products are often subjected to high temperatures in their daily use, this lack of thermic stability of the EGCG molecule could limit the development of EGCG-based skin products. (Table I)

pH Value

Another parameter influencing the stability of EGCG is the pH value of the solution EGCG is solved in. In a 2006 study, different EGCG solutions were subjected to different pH values. The pH-values varied from 1,6 to 9. The solution at 1,6 was stable for 4 days, the solution at 9 only for 4 hours. Higher pH values seemed to directly affect the molecule stability. (Radhakrishnan et al., 2016)

These findings were consistent with a 2003 study where EGCG in a buffer with a physiological pH of 7,4 degraded almost fully after 6h. Similar results have been reported for EGCG in water at 100 °C. (Su et al., 2003)

Li at al. took a closer look at the stability of the molecule at high temperatures compared to pH values. EGCG heated at over 100 °C for 30 minutes was reported to have better stability at pH between 3,8 and 5. (Li et al., 2012)

The molecule stability was reported to be very good at pH <4, good at 4 < pH < 8 and poor at pH > 8, results once again consistent with the existing literature. (Li et al., 2012; Radhakrishnan et al., 2016; Su et al., 2003; Zhu et al., 1997)

EGCG in a buffer of 7,2 pH has been shown to react with O2 and lead to the building of superoxide radicals which can contribute to the degradation of EGCG itself. (Sang et al., 2007) (Table I)

Table 1: Description of the effects of temperature and pH value on EGCG-stability.

RT = room temperature; HL = half-life; C = concentration; R = reaction; E = parameter that mostly effects EGCG-degradation; S = stable for; ↓ = decrease; ↑ = small increase; ↑↑ = medium increase; ↑↑↑ = large increase. Table I has been already published in Frasheri et al. 2020, we received permission from the author to include it in our publication

Parameters	Study	Concentration	Setting/Study design	Outcome
Concentration	(Sang et al., 2005)	0,51 mM	RT for 7 days	20% ↓ in C
		2,06 mM		20% ↓ in C
		6,99 mM		No change
		20 µM	HL in water at 37°C	30 min
		96 µM		150 min

	(Fangueiro et al., 2014)	900 µg/mL	2 days at 4°C	~0% ↓
		25 µg/mL		~40% ↓
		0,25 µg/mL		~100% ↓
	(Li et al., 2012)	1666,7 µg/mL	At 80°C for 6h	14,66% ↓
		5 mg/mL		43,13% ↓
	(Zeng et al., 2018)	2 mg/mL	At 25° to 28° C	~20% ↓ in C
		0,5 mg/mL		Fully degraded
		0,1 mg/mL		Fully degraded
Temperature	(Fangueiro et al., 2014)	900 µg/mL	C at 4°C – C at RT	~20% ↓
		25 µg/mL		~75% ↓
		0,25 µg/mL		Both fully degraded
	(Li et al., 2012)	1666,7 mg/mL	in Green Tea Concentrated Solutions at neutral pH	t_{90}c at RT = 27797 min
				t_{90}c at 120°C = 3,273 min
	(Wang et al., 2008)		<44°C	R: Oxidation ↑↑↑
			>44°C; <98°C	R: Epimerization ↑↑; Oxidation ↑
			>98°C	R: Epimerization ↑↑↑
pH-value	(Proniuk et al., 2002)		>4°c; <25°C	E: Temperature
			>25°C	E: Temperature and pH- value
	(Radhakrishnan et al., 2016)		pH 1,6	S: up to 96h
			pH 5	S: 48h
			pH 7	S: 24h
			pH 9	S: 4h
	(Zhu et al., 1997)		pH < 4	Great stability
			4 < pH < 8	Poor stability
	(Li et al., 2012)		Stability after pre-heating at 120°C for 30min	3,8-5 pH → highest stability
	(Su et al., 2003)		pH 7,4	After 6h completely degraded
			100°c	

(Frasheri et al., 2020)

Dermatological applications

The application of EGCG in dermatological and cosmetic products has been complicated by its instability at high temperatures. (Fangueiro et al., 2014; Li et al., 2012; Proniuk et al., 2002; Wang et al., 2008)
Scalia et al. 2013 examined the interaction between EGCG and Vitamin C/E, BHT and alpha-lipoic acid. The scientific purpose was that of establishing whether the antioxidants were able to improve EGCG stability. The creams were irradiated using simulated sunlight for approximately 60 minutes and the remaining EGCG concentrations were examined and compared to a concentration of the cream containing only EGCG (baseline). The creams containing EGCG plus BHT and Vitamin E showed lower EGCG percentages than the baseline (15,5% and 21,9% compared to 23,1%). The creams containing EGCG plus vitamin C and alpha-lipoic acid showed higher EGCG percentages than the baseline (79,6% and 87,4% compared to 23,1%). Additionally, the scientist performed antioxidative activity tests and vitamin C and alpha-lipoic acid performed better than the other two antioxidants. (Scalia et al., 2013)
When compared to enteral delivery, topical delivery was reported to perform better. EGCG was applied to the back of skinless mice. For the epidermis, concentrations of 1365 ng/mL and half-life of 9 hours were reported, for the dermis, 411,2 ng/mL and 11 hours. The gel used for the testing was subjected to

temperatures ranging from 4 to 37 degrees Celsius and once again it was reported that the molecule was less stable at higher temperatures. (Lambert et al., 2006)
EGCG in emulsion and hydrogel form were tested in a 2014 study with the purpose of comparing their ability to penetrate the skin. Similar concentrations of both delivery forms were reported in the skin, the emulsion seems to reach mostly the deeper skin layers, whereas the hydrogel remained more superficial. The hydrogel was also reported to have better stability. (Scalia et al., 2014) (Table II)

Table 2: Description of the effects of some dermatological ingredients on EGCG stability and effects of topical EGCG-application on Dermis and Epidermis

C = concentration; HL = half-life; ↓= decrease by
Table II has been already published in Frasheri et al. 2020, we received permission from the author to include it in our publication

Parameter	Study	Specific parameter	Setting	Outcome
Cosmetic ingredients	(Scalia et al., 2013)	EGCG only	oil-in-water emulsions after 1h irradiation	76,9% ↓
		EGCG + Vitamin E		79,1% ↓
		EGCG + BHT		84,5% ↓
		EGCG + Vitamin C		20,4% ↓
		EGCG + alpha-lipoic acid		12,6% ↓
	(Bianchi et al., 2011)	ethylhexyl methoxycinnamate		61 % ↓
		butyl methoxydibenzoylmethane		69% ↓
Topical application of 28,6 µg/cm²	(Lambert et al., 2006)	Epidermis		C: 1365,7 ng/mL; HL: 9,3h
		Dermis		C: 411,2 ng/mL; HL: 10,9h

(Frasheri et al., 2020)

Interactions with cosmetic ingredients

Most sunscreens contain multiple molecules which interact with each other and influence the UV-protecting properties of the product. Literature research focusing on the interactions between EGCG and cosmetic ingredients was therefore deemed necessary before the developing the sunscreen.

The interactions of EGCG with common cosmetic ingredients have been shown to be beneficial in the enhancement of both EGCG and the cosmetic ingredients properties.

Vitamins

Vitamin A is used in both the dermatologic treatment of acne vulgaris and the cosmetic treatment of photoaging and wrinkles. ATRA is a vitamin A derivate and has been reported to enhance the effect of EGCG and help inhibit the development of melanoma in mice cells. (Lee et al., 2010)
Vitamin C has similar cosmetic properties to vitamin A. As already reported in the stability subsection vitamin C has been shown to enhance EGCG stability (and prevent UV damage) and help preserve its anti-oxidative effects. (Scalia et al., 2013)
The molecules seem to interact both ways, as EGCG has been shown to improve the antioxidative abilities of both vitamins. (Intra & Kuo, 2007)
Despite not improving EGCG stability, vitamin E (specifically α-Tocopherol) interacts with EGCG in the prevention of linoleic acid hydroperoxide endothelial cell damage. (Kaneko et al., 1998; Scalia et al., 2013)

UV filters

Titanium dioxide (TiO_2) is a molecule that can be found in many cosmetic products. Its role in sunscreen products is further investigated in the "environmental impact of sunscreens" subsection.
In most sunscreen products the molecule is present in its nanometer form which protects the skin by absorbing sun-light and builds a superficial sunscreen film. The molecule can also be found in its pigment form in many foods, toothpaste and some medications. (cosmeticsinfo, 2019)
Verma et al. looked at the interaction between the polyphenol and titanium dioxide. Green tea extracts coated with the UV-filter led to lower photocatalytic activity, making its cosmetic application in sunscreen easier and more accessible. (Verma et al., 2016)
As previously mentioned under the section "stability" butyl-methoxydibenzoylmethane did not provide for better EGCG stability. Benzophonene-4 was shown to reduce the photo-degradation process by 17%. (Bianchi et al., 2011)

Hyaluronic Acid

Hyaluronic acid (HA) is used in many common cosmetic skin-hydrating products.
The interaction between hyaluronic acid and EGCG has been studied in 2017. EGCG/HA transferosomes were reported to have higher antioxidative properties and to better penetrate and deposit in the skin. (Avadhani et al., 2017)

Isopropyl myristate

Isopropyl myristate is used in cosmetic products due to its ability to facilitate cutaneous penetration.
The interaction between the molecule and the 4 most common green tea polyphenols showed that EGCG had the second-best drug-loading capacity and release time. Having optimal drug loading capacity and release time is essential for the manufacturing of a cosmetic emulsion. (Chaiittianan & Sripanidkulchai, 2014)

Butylated hydroxytoluene

As previously mentioned, BHT was not able to increase EGCG stability after 60 minutes under simulated sunlight. A previous study had already taken a look at the molecule stability under different thermic conditions. A hydrophilic ointment of the polyphenol with 0,10% butylated hydroxytoluene was tested at 25 and 37 degrees Celsius. After respectively over 4 months and 6 months approximately 10% of EGCG was lost due to degradation. The same degradation was reported for the EGCG-ointment (without BHT) after 7 days. (Dvorakova et al., 1999)

Environmental impact of sunscreens

The goal of the study, in the development of the EGCG-based sunscreen, was that of not using microplastics and only using mineral UV-filters in microparticulate form.

Microplastics

Microplastics are plastic particles smaller than 5 mm in diameter. There is a distinction between primary and secondary microplastics. Primary microplastics are created for commercial use in, for example, sunscreens and are purposely made to be smaller than 5 mm in order to provide the cosmetic/sunscreen products with better consistency and galenic. Secondary microplastics are the result of the degeneration process of bigger plastic products, for example plastic bottles.(Liitschwager, 2019) The pollution of sea and oceans due to secondary microplastics has been the object of scientific and media scrutiny for decades and has led to multiple initiatives and to the creation of non-profits which deal with the problem, such as The

Ocean Cleanup. The effects of primary microplastics on the environment have only recently become the topic of scientific discussion.

It has been shown that microplastics cause oxidative stress and damage to marine living organisms. Furthermore, it has been reported that they might have neurotoxic and genotoxic effects. (O'Donovan et al., 2020)
Microplastics can also enhance the toxic effects of nanoparticulate titanium dioxide and cause oxidative stress in the form of reactive oxygen species and lipid peroxidation in marine algae. (Thiagarajan et al., 2019)

UV-filters

UV-filters can be also divided into two groups:

- Mineral/physical filters: they reflect UV-radiation away from the skin and work as a barrier between UV-light and stratum corneum. Common examples are TiO2 and ZiO
- Chemical filters: they absorb UV-light and turn it into heat. Common examples of are oxybenzone, avobenzone, ecamsule, octisalate, octinoxate

Many chemical UV-filters have been found in high concentrations in water of seas and oceans all over the world. A 2014 study measured the levels of organic filters in surface water of Hong Kong, Tokyo, Bangkok, New York, Los Angeles, Arctic, Shantou and Chaozhou. Benzophenone-3 (BP-3) and Ethylhexyl methoxycinnamate (EHMC) were found in all tested areas and very high concentrations were reported even in recreational areas. (Tsui et al., 2014) BP, BP-1 and 3 have also been found in the urine, semen and serum of young Danish men. (Frederiksen et al., 2021) EHMC has been reported to have an estrogenic and/or antiandrogenic effect on fish (Pimephales promelas). EHMC was also shown to induce changes in the testicles and ovaries of the fish, which had respectively fewer spermatocytes and previtellogenic oocytes.(Christen et al., 2011)
Chemical UV-filters have also been shown to cause Type IV contact allergies. (Schauder & Ippen, 1988)

Ultraviolet skin damage

UV-light can have positive and negative effects on the organism. On the one hand it plays a key role in the transformation of 7-Dehydrocholesterol into cholecalciferol, which is then processed into becoming vitamin D. UV-light is also used therapeutically in the dermatological field in the treatment of common skin conditions such as atopic eczema, psoriasis, mycosis fungoides and many more. On the other hand, UV-light exposure can be the cause of skin cancer and trigger many other conditions such as bullous pemphigoid or lupus erythematosus. In addition to the more clinical consequences of UV-induced skin damage, it should also be mentioned how UV-light (in particular UV-A) accelerates the aging process in the skin. The easiest way to protect the skin against ultraviolet-light-induced damage is the daily application of sunscreen products. The purpose of this study was that of developing a sunscreen product which, in addition to being environmentally friendly (not containing microplastics, nanoparticles or chemical UV-filters) could provide a Sun Protecting Factor (SPF) of 50.

Health aspects

The health aspects of EGCG are well reported and vary from antioxidant to antiangiogenic and anticancerogenic. (Zink & Traidl-Hoffmann, 2015a) In the literature research an important role was given to the therapeutic effect of EGCG in the treatment of common dermatological conditions. In particular the application of the molecule in the prevention and treatment of UV-induced skin damage was carefully assessed, as it is the scientific basis for the development of the EGCG-sunscreen.

Skin cancer and UV damage

When it comes to both UV-A and UV-B induced skin damage, EGCG has been reported to protect the skin against UV-rays which can lead to both skin-aging and different types of skin cancer.
A 2001 study analyzed the effects of UV-A damage on both mice and cultures of human dermal fibroblasts. 1 day after EGCG application a reduction in both collagen synthesis (in the animals) and collagenase secretion (in the cultures) was reported. (Kim et al. (2001)
In the same study it was also tested for UV-B-caused redness (erythema) on guinea pigs, where EGCG was reported to reduce the redness on the 24-h-post-irradiation mark. (Kim et al., 2001)
Langerhans cells of the epidermis are often damaged by UV-light. In order to test the molecule's ability to prevent such damage, human skin was treated with different EGCG concentrations ranging from 1% to 10%. The histological samples displayed 58% more Langerhans cells in the treated skin compared to the control (vehicle). The EGCG-concentrations above 2,5% performed the best results, these findings are consistent with the previously mentioned molecule dose-dependency. (Elmets et al., 2001)
Other important biochemical markers for UV-induced damage are CD1a+ cells depletion and 8-OHdG (anti-8-hydroxy-2´-deoxyguanosine) formation. Both were tested in a 2009 study where non-irradiated and irradiated skin was compared after both vehicle and EGCG application. The irradiated and EGCG-treated skin displayed 35% and 57% higher CD1a+ cells than respectively the unirradiated untreated skin and the irradiated vehicle-treated skin.
The differences in 8-OHdG levels were not significant, but it should be noted that the levels, albeit non-significant, were lower for the vehicle-treated skin. (Camouse et al., 2009)
Hydrogen peroxide and nitrous oxide in the cutis are also two common byproducts of UV-induced damage. Their production and the infiltration of CD11b+ cell (another marker of oxidative stress) can be diminished by EGCG. The same can be said for LPO production (epidermal lipid peroxidation), EGCG is also been reported to enhance GSH (an antioxidant) production. (Katiyar, Afaq, et al., 2001)
Another mechanism by which EGCG might reduce skin damage might be suppression of both mitochondrial dysfunction and keratinocytes apoptosis. (Liu et al., 2016)
Probably the most common light induced skin damage marker is cyclobutane pyrimidine dimers (CPD). EGCG has been reported in a test on human volunteers to partially block cutaneous UV-penetration and therefore inhibit CPD formation, the effect of the molecule showed dose-dependency once again. (Katiyar, Bergamo, et al., 2001; Katiyar et al., 2000)
On mice EGCG has been shown to lead to 60% lower cancer incidence, 86% lower cancer multiplicity and 95% lower overall cancer growth than the control group. The transformation from benign papillomas to malign carcinomas has also been reduced by ca. 80%. (Katiyar et al., 2007; Mittal et al., 2003)
In a 2002 study in mice (mice irradiated with UV-B for 20 weeks and treated with EGCG for 18 weeks) EGCG was reported to lead to higher cell apoptosis in not malign tumors (72%) and in squamous cell tumors (56%). In hyperplastic areas and areas not affected by tumors no improvement could be detected. (Lu et al., 2002)

Psoriasis

The etiology of psoriasis is very complicated and multiple factors contribute to it. Being a chronic inflammatory skin disease, it makes sense that EGCG might be used in its treatment, due to the molecule's anti-inflammatory abilities.
A 2016 study analyzed these abilities in the treatment of psoriasis-like-dermatitis in mice. The dermatitis had been induced using Imiquimod, a medication normally used in the treatment of HPV-warts, which has also been shown to cause these psoriatic efflorescences in mice. (van der Fits et al., 2009; Zhang et al., 2016)

After the first 6 days the early intervention group showed only mild symptoms (some redness, thin scales, no visible infiltration). The medium-term intervention group showed to be more symptomatic at the end of the second day (redness, scales and infiltration). After EGCG application on day six the symptoms got milder and were comparable to the ones of the early intervention group. (Zhang et al., 2016)
A green tea extracts solution (with 40% EGCG) was used in the treatment of flaky skin mice.
Compared to the control group (only treated with water), the intervention group presented skin lesions a week and a half later, the lesions were also not as severe. This discrepancy could also be confirmed using histological skin samples. The histological samples of the intervention group presented milder histological findings than the ones of the control group. (Hsu et al., 2007) EGCG in a nanometer formulation was also seen to be 20-fold more effective than the normal EGCG molecule. (Chamcheu et al., 2018)

Human papillomavirus

The human papillomavirus (HPV) is well-reported to play a key-role in the development of cancer of the cervix. Not every HPV strain has a carcinogenic effect, many lead merely to the development of palmar, plantar or anogenital warts (also known as condylomata acuminata). EGCG is known for its role in the treatment of HPV-warts as the first medical product on the market containing EGCG was indeed approved against condylomata acuminata.
An ointment containing different green tea polyphenols, amongst which EGCG was the most abundant, has been tested on individuals showing from 2 to 30 HPV-warts on the ano-genital area.
When compared to the control, the intervention group was reported to have a two-fold higher warts clearance rate. The reoccurrence rate was comparable for both groups. (Tatti et al., 2010)
Two 2007 and 2008 and study reported similar results. (Gross et al., 2007; Stockflth et al., 2008) The three studies displayed differences in time needed for clearance and gender specific clearance results. (Gross et al., 2007; Meltzer et al., 2009; Stockflth et al., 2008)

Atopic dermatitis

Given the chronic inflammatory nature of atopic dermatitis, it comes as no surprise that the anti-inflammatory properties of EGCG have been tested in the treatment of the disease.
The molecule has been reported to help with common atopic dermatitis symptoms, such as redness, edema, excoriation and scaling (mice studies). (Noh et al., 2008)
On humans an EGCG product has been shown to alleviate some moderate symptoms on the head and neck of the volunteers after a week. The 2005 double blind randomized clinical trial reported some interesting results, especially on the lesions on the neck of the volunteers, the findings were, however, non-significant. (Patrizi et al., 2016)
Bath therapy plays a key role in the daily treatment of many atopic dermatitis patients. A green tea extracts bath therapy displayed significant improvement in the SCORAD of the patients. The visual analog Scala data were however non-significant. (Kim et al., 2012; Zink & Traidl-Hoffmann, 2015a)
The possible treatment of atopic dermatitis with EGCG and green tea extracts requires further investigating and scientific research.

Alopecia

In a 2011 study on mice EGCG application leads to a hair-loss reduction by reducing the apoptosis induced by testosterone. The molecule seems to also improve the regrowth of previously epilated hair. (Kim et al., 2011)

It has been speculated that the reason for this reduction in hair-loss might rely on the inhibition of the key molecule which leads to androgenetic alopecia, 5-α-reductase. This inhibition is the pathogenic basis of the most common hair-loss products on the market and seems to be induced by EGCG. (Hiipakka et al., 2002) EGCG seems to show positive effects even on volunteers not suffering from androgenetic alopecia. Cells in the papillar dermis on the head of human volunteers showed to be positively affected by an EGCG solution. The results are consistent with the existing literature and the previous in vitro studies. (Kwon et al., 2007)

Skin flaps

Skin flaps are commonly used in many dermatologic and plastic operations. One of the main post-operatory issues is skin flap necrosis.
To assess to possible role of EGCG in flaps necrosis prevention, EGCG was applied on skin flaps in mice both topically and injected in the flap itself and then compared to a vehicle. The topically applied polyphenol provided for better regional blood perfusion, VEGF expression, capillary density and overall survival than the vehicle. Injected and topically applied EGCG performed similarly, the topical version performed better in the "blood perfusion" department. (Cheon et al., 2012)

In-vivo testing

After developing a sunscreen which met all the required criteria listed below:

- No microplastics
- No nanoparticles
- 100% biodegradable
- SPF of 50
- Containing EGCG

The focus of our project shifted to invivo testing.
Four different prototypes of an EGCG sunscreen were developed and further tested. A total of 104 volunteers were recruited and, after written consent was obtained, they were provided with the sunscreens in order to pick 1 out of the 4 for further testing.
The sun protecting properties of the sunscreen were tested in vivo on 10 volunteers using solar simulators and MED-tests for both UV-A and UV-B rays and then the cutaneous UV-induced reactions were quantified using a Chromameter.

The sun-protecting factor is generally calculated using the skin response to only UV-B rays, but UV-A was included because this wavelength can be responsible for premature skin aging, indirect DNA damage and antioxidative stress. (Amaro-Ortiz et al., 2014) After UV-B and UV-A radiation, the vascular reaction of the skin by way of Raster Scan Optoacoustic Mesoscopy was quantified. (Hindelang et al., 2019)

The anti-inflammatory properties of EGCG were investigated over a 28 days period on 33 volunteers using Transepidermal water loss (TEWL) and Stratum corneum hydration (SCH) tests. It was decided to test the sunscreen's ability to increase the cutaneous hydration levels and to minimize transepidermal water loss by improving the protective effects of the superficial skin layers. At the end of the 4 weeks period, volunteers' satisfaction and overall opinion of the sunscreen product were quantified by way of questionnaires.

The EGCG molecule tends to oxidate very quickly, this biomolecular process becomes apparent when the color of EGCG solutions turns brown. (Krupkova et al., 2016; Sang et al., 2005) In order to test whether the sunscreen product would leave traces of brown pigment on textiles white T-shirts test were performed.

The European standard for sunscreen application is 2 mg/cm², which means that the average German adult would have to apply 38 grams of sunscreen to be fully protected. (Bundesamt, 2017) Based on the existing scientific data this amount was believed to be unrealistic, the in-vivo doses of sunscreen applied by the volunteers were tested and compared to the European standard. (Williams et al., 2018)

The ”Bundesministerium für Wirtschaft und Energie” hast approved and financially supported the entire project through “das zentrale Innovationsprogramm Mittelstand (ZIM)”.

Objective

The aim of this study was to develop a sunscreen based on epigallocatechin gallate, leveraging the protective effect against UV-light and its advantage of anti-inflammatory properties of the green tea polyphenol without the use of microplastics and mineral UV-filters exclusively in microparticulate form. Furthermore, to assess the developed sunscreen in addition to its UV protection and anti-inflammatory effect in terms of patient preferences, water loss and hydrations levels of the skin.

Scientific review

Before starting with the development of the sunscreen, a detailed literature research was deemed necessary. The literature research process led to the publishing of a review with the title “Great green tea ingredient? A narrative literature review on epigallocatechin gallate and its biophysical properties for topical use in dermatology” in the journal Phytotherapy research.
The review was written with the goal of examining EGCG, its stability under different temperatures, pH-values, concentration, its interaction with other typical cosmetic molecules and its possible dermatological therapeutic effect. These endpoints were set with the goal of collecting data, which could later be used for the development of the EGCG-sunscreen.

Amongst the many polyphenols that can be found in *Camellia sinensis,* EGCG has been shown the be the one with the highest concentration. The properties of the molecule are well reported and have shown to be effective against angiogenesis, microbial pathogens and inflammation. (Chakrawarti et al., 2016)

EGCG has already been used in the development of many dermatological products, it has not yet seen a widespread dermatological application, mostly due to its poor stability under natural conditions like medium to high temperatures. The pH value of the skin has also been shown to not be ideal for the stability of the molecule.

The existing literature was examined looking for cosmetic ingredients that could therefore improve the stability of the polyphenol.
One of the goals of the review was looking at the therapeutic application of EGCG in the treatment of common dermatological diseases like HPV-warts and light-induced biochemical dermal damage.
The effects of pH-value, temperature, concentration and the solution in which the molecule is submerged have been examined in relation to the molecule stability. Possible stability enhancement due to interaction with other molecules has also been studied. EGCG showed poor stability even before being extracted from the green tea leaves. Commercial tea bags left at 20°C for 180 days showed a 28% decrease in EGCG concentration. (Friedman et al., 2009)
One of the reasons for the polyphenol lack of stability is the different chemical reactions that can lead to production of different chemical products. EGCG in water has been shown to oxidate and therefore change the color of the medium to brown. Epimerization can be seen at very high temperatures and very low pH-

values, where EGCG turns to its trans-epimer GCG (Krupkova et al., 2016; Wang et al., 2008)

Development of a biodegradable, eco-friendly EGCG-sunscreen

Two very common ingredients which can be found in most sunscreen products are nanoparticulate titanium dioxide (TiO2) or zinc oxide (ZnO). These two molecules are mineral (or physical) UV-filters and they work by blocking and reflecting UV-rays. Chemical UV-filters are absorbed by the skin and do not reflect UV-rays, but they disperse them as low energy heat. Benzones are common chemical UV-filter. The previously mentioned mineral UV-filters TiO2 and ZnO in nanoparticulate form have been shown to lead to DNA damage and have neurotoxic properties, which might cause epilepsy, Alzheimer's and autism. (Mohamed & Hussien, 2016; Notter et al., 2018; Song et al., 2015)

The reason why nanoparticulate titanium dioxide and zinc oxide are preferred to their microparticulate forms, despite their potential damage to the environment, relies on the cosmetic advantage the nanoparticles provide. Bigger particles (microparticles) build a white cast on the skin after application, which is seen by many consumers as esthetically unpleasant and has lower market acceptance.

In addition to nanoparticles, many sunscreen products also contain microplastics. These significantly increase water resistance of the sunscreen products and provide for more stable microemulsions. Because of widespread sunscreen usage, these microplastics are often released into the environment and the oceans in quantities that are difficult to estimate. Microplastics can therefore find their way into the food chain of aquatic organisms and ultimately also into that of humans.
For these reasons, the state of Hawaii has decided for the ban of the sale of numerous conventional sun creams that contain environmentally harmful substances from January 1, 2021. Many UV filters contained in sun protection products, such as octinoxate and oxybenzone have also been found in coral reefs and in various fish species; this could have consequences for the food chain and thus also impact humans. (Altmeyer & Barth, 2020)
For the previously listed reasons it was decided to create a sunscreen which takes advantage of the anti-inflammatory and sun blocking properties of EGCG while being biodegradable, containing only natural additives and not using any microplastic or nanoparticles. For the development of the sunscreen mineral UV-filters have been used in their microparticulate form.

Questionnaires and selection of one sunscreen

Four different versions of the EGCG-sunscreen were developed with the cooperation partner SystemKosmetik. Each version had a different consistency, galenic and color, they had all previously been lab-tested for their UV-Light protection (SPF 50) and all contained EGCG-extracts. Only one of the 4 sunscreens had to be selected to be later produced in bigger quantities and be available for the next tests. To choose the most consumer-friendly out of the four sunscreens, it was decided to develop a questionnaire, that would help us quantify consumer response to each of the four products.

Ultraviolet light, MED and chromameter

After selecting one of the 4 sunscreens Systemkosmetik, proceeded to develop 40 versions of the selected sunscreen and shipped them to the department of Dermatology and Allergology of Klinikum rechts der Isar. The UV-B and UV-A protection had already been lab-tested and the in-vivo tests on willing participants could therefore start.
The aim of the study was to test UV-protection against both UV-B and UV-A light. Many products on the market are only tested for UV-B protection. UV-A radiation has been shown to accelerate the skin aging

process. The aim of the test was therefore to not only quantify the ability of the sunscreen to protect from skin cancer, but also from skin aging. (Svobodová et al., 2012)

Minimal erythema dose (MED) tests were performed to assess UV-B and UV-A protection.

The chromameter was then used to quantify the redness/hyperpigmentation reactions at hour 0 and 24 after UV-exposure.

Raster scan opto-acoustic mesoscopy

RSOM has been used to examine and evaluate the protective effect of EGCG. For this purpose, several areas of the skin (protected and unprotected) have been examined after exposure to UV-A and UV-B light. The extent and depth of the UV-induced vascular response in with-EGCG-protected skin is compared to the vascular response in non-protected skin.

Transepidermal water loss and Stratum Corneum Hydration

Based on the preexisting evidence showing the antioxidative and anti-inflammatory properties of EGCG and other green tea extracts, the goal of the tests was that of quantifying whether the sunscreen could improve or worsen the hydration level and the barrier function of the human epidermis. To do so Stratum Corneum Hydration (SCH) and Trans Epidermal Water Loss (TEWL) measurements were used.

Questionnaires after TEWL and RSOM

At the end of the 28 days long TEWL and STH tests to provide the participants were provided with questionnaires to evaluate the overall subjective response to the sunscreen now that they had had a chance to incorporate the product in their daily skincare routine.

Brown-coloring tests

The intensive scientific research with special emphasis on EGCG stability and degradation has shown that the enzyme polyphenol oxidase can be responsible for oxidation and thus for the brown coloring of skin and textiles, which can often be seen after using EGCG products. To avoid this common problem, this oxidation process was stopped at the molecular level by means of microencapsulation.

The EGCG used in the formulation was obtained naturally from the leaves of green tea. The leaves were first extracted in water and then went through a "pure water double infusion" process. Then it was carefully steamed or air-dried. A nearly decaffeinated, highly pure product was obtained (> 94% EGCG; <0.1% caffeine).

After the microencapsulation of the EGCG particles in the manufacturing process, an evaluation of possible brown coloring by the product on both the skin and on textiles was carried out and quantified.

Sunscreen application dosage

According to the European standards, approximately 2 mg of sunscreen should be applied per square centimeter of skin to provide for an accurate UV-B protection. This is the standard by which all the major sunscreen companies test their products to make sure that UV-B protection is provided. The average adult BSA is 1.7 m^2 (1.9 m^2 for males and 1.6 m^2 for females). It is quickly apparent how this standard of 2 mg/cm^2 is not realistic for most human beings. An average adult would need 34 g of sunscreen to apply enough on his/her body.

The amount of sunscreen the participants in the T-shirt-tests used, was compared to the “correct” amount according to the European standards.

Materials and methods

Scientific review

Literature research and databases

For the literature research published journal articles were considered (clinical trials or scientific reviews). Studies were identified by searching electronic databases (PubMed and MEDLINE) and reference lists of respective articles. Because of the lack of previous reviews, which examined the three main endpoints, all articles disregarded their year of publication were considered eligible. Multiple searches took place during the writing process, the last of which was on 26th of January 2020.

Eligibility criteria

Due to the existence of three different endpoints, different eligibility criteria were applied to each of them. For the stability subsection, only literature examining the unaltered molecule (meaning non-coated EGCG) was examined.
For the interactions subsection, the research was limited to common cosmetic ingredients. With the help of a board-certified dermatologist a list of common ingredients, found in most cosmetical products, was put together. (cosmeticsinfo, 2019)
Due to the more than abundant literature examining possible health application of EGCG, the focus of the health aspects subsection was only on common dermatological diseases. Intravenous or enteral use were not considered in the literature research, only direct topical application.

Development of a biodegradable, eco-friendly EGCG-sunscreen

In order to fulfill all the requirements, various suppliers for mineral UV-filters were contacted. The composition, particle size, distribution, shape and purity of these raw materials were carefully examined and tested. The requirements and exclusion criteria (e.g., no nanoparticles) are listed in the subsection “objective”. The selected raw materials were then incorporated into the formulations based on the previous calculation of the SPF. In these tests, the focus was the incorporation of the UV-filters in the formulation (in order to maintain their properties), the degree to which it was possible to incorporate said filters in the formulation, what manufacturing conditions were necessary and, above all, whether the formulation was able to stay stable and not degrade. The best results were obtained with the Zano® products (zinc oxides) from EverZinc, based on which Xperse® type (dispersion of Zano® in Caprylic / Capric Triglyceride) was chosen. It was also ensured that the maximum permissible concentration of zinc oxide (25.0%) was not exceeded.

Various calculations of the necessary concentration of the substances were carried out in Silico using the SunScreen Simulator (https://www.sunscreensimulator.basf.com/Sunscreen_Simulator/login). Then different concentrations were mixed and sent to an external institute for a confirmatory measurement for SPF screening. The desired SPF of 50 was achieved at a concentration of 18% titanium dioxide and 18% zinc oxide.

In order to find a stable basic formulation that meets previously mentioned criteria, numerous formulation attempts were made on the basis of natural polysaccharides, which were subjected to a stress/stability test. The formulations were first observed in neutral glass for a few weeks at room temperature in order to determine any optical change under normal conditions. The formulations were then additionally stored at +40 ° C and -15 ° C in order to be able to rule out changes in the event of thermal stress, which are very common for sunscreen products. The formulation was observed daily for a week. If there was no change

(color, smell, consistency, stability, etc.), the observation period was extended by one more week. If there were changes, the formulations were adjusted accordingly and tested again as described above. (figure 1 and 2)

Figure 1: Unstable solutions; change in formulation color (white to brown) after 1h at room temperature

Figure 2: stable formulations; no change after multiple weeks at room temperature

Questionnaires and selection of one sunscreen

104 participants willing to test the sunscreens on their own skin were recruited and provided with the 4 different versions of the product and one questionnaire each. In order to not influence the results of the survey the participants were provided with only the following information:

1. Each sunscreen contains Epigallocatechin gallate (it was shortly explained what EGCG is and where it comes from)
2. Each sunscreen differs from the others in its consistency, galenic and color.
3. Every sunscreen is 100% biodegradable

2 out of the 4 products looked like “normal” sunscreens, in that they had the typical white color that most sun-blocking products have, the other 2 had a brown color which is typical for products containing EGCG since the molecule tends to oxidize very quickly and to turn brown. This quality of EGCG has been further examined under the subsection “stability” of the review and how this brown color might affect the marketability of the product in the in vivo “T-shirt-tests”. Participants were not provided with information concerning the reason for the different colors as to not to influence their choices in any way.

Each participant was asked to put all 4 sunscreens on. In order to avoid confusion once the creams had been absorbed by the skin, they were advised to put sunscreen 1 to 4 clockwise on respectively left upper arm, left lower arm, right lower arm and right upper arm and to make sure that creamed areas did not overlap. That would help them differentiate each sunscreen and make for more accurate test-results. After the application of the sunscreen the participants were provided with a questionnaire and a pen. The time to complete the survey ranged from 10 to 30 minutes per participant. At the end the questionnaires, which had been previously pseudonymized to guarantee the anonymity of the participants, were collected. Each participant was assigned a number, a private list with the names of the participants and their corresponding test-number was kept in case one of the participants decided on a later date to revoke their informed consent. The questionnaires were only marked with the test numbers. The entire project including the in-vitro and in-vivo was subjected to an ethical vote and approve by the ethical commission of the Technical University München.

The questionnaires consisted of 42 questions, 22 of which aimed to collect epidemiological data regarding not only age, sex and occupations, but also sun-protection habits and personal sunscreen preferences of the participants. 20 questions aimed to directly evaluate and quantify which one of the 4 sunscreens the participants preferred. The EGCG-sunscreen-specific questions ranged from overall satisfaction to likelihood to buy the product and subjective feeling of sunscreen protection. In the results some of the most interesting findings with regard to both the “epidemiological” and “EGCG-specific” questions have been listed. The questionnaires were designed in collaboration with SystemKosmetik, they were then validated by a team of dermatological experts and were provided to the participants pseudonymized and in paper form.

It took us approximately 1 month to recruit the 104 volunteers and collect the data.

All the data was imported and elaborated using IBM SPSS Statistics 26. To make the results easier to understand the SPSS Table results have been sometimes converted to vertical bar or pie charts.

UV-B and UV-A Minimal erythema dose (MED) and Chromameter

The MED-tests are a standardized procedure used routinely at the Department of Dermatology of Klinikum rechts der Isar, where all the tests took place. The MED-tests are carried out in the range of UVB-311 nm and UVA-1 in a non-pre-tanned area of the patient's skin (usually gluteal, the dorsal thigh was used instead because of space reasons). Using a template, 6 small areas of skin are irradiated with UV rays (UV-B radiation in the range of 311 nm, UVA radiation in the range of 340 to 400 nm) in increasing doses (UVB-311nm: 25 to 250 mJ / cm^2, UVA-1: 5 to 25 J / cm^2). This allows the individual minimum erythema dose (MED; erythema = skin reddening) to be determined. The minimum erythema dose is the lowest dose of UV light that leads to a visible reddening of the skin. The erythema is measured for the first time 24 +/- 2 hours after the irradiation. The irradiated region must not be treated with cortisone creams until the reading on the following day, in order not to influence the local skin reaction.

This procedure is carried out for both a sunscreen-covered and a sunscreen-free skin area and the SPF is calculated using the following formula.

SPF = MED with light protection agent / MED without light protection agent

When a product with SPF 50 is applied, it will protect the skin until it is exposed to 50 times more UVB radiation than it would be required if there was not any UV-protection. So, if it were to take 10 minutes for a person with a specific skin type to get burnt under a specific intensity of UV-light (Fitzpatrick skin type I), an adequately applied sunscreen with SPF 50 would provide protection for approximately 500 minutes (10

minutes x 50 = 500 minutes). UV-A and UV-B Minimum Erythema Dose (MED) tests were carried out in order to test the UV-A and UV-B protection of the sunscreen in vivo.

Method

10 willing participants, 5 men and 5 women were recruited to provide for gender-balanced results. All the participants had either a I or II Fitzpatrick skin type. 4 different MED-tests were carried out on each participant:

1. UV-B MED on an unprotected skin area
2. UV-B MED on a skin area protected with sunscreen
3. UV-A MED on an unprotected skin area
4. UV-A MED on a skin area protected with sunscreen

The UV-B MED consisted of 6 radiation fields, each field was continuously irradiated with artificial UV-B until a specific mJ/cm² dose was reached and messed with the dosimeter. After reaching the previously selected dose the cap of the irradiation field would automatically close and therefore not receive any more UV-B radiation. The radiation fields and their UV-B dose are listed here:

- 1. Field = 25 mJ/cm²
- 2. Field = 50 mJ/cm²
- 3. Field = 75 mJ/cm²
- 4. Field = 100 mJ/cm²
- 5. Field = 125 mJ/cm²
- 6. Field = 150 mJ/cm²

The UV-B MED-tests were carried out on the left dorsal thigh of the volunteers. Normally MED-tests would be carried on the gluteal region, because of space reasons both UV-B and UV-A MED-tests had to be carried out on the left (for UV-B) and right (for UV-A) dorsal thighs. The first MED-test was carried out directly on the skin of the participants and the second one on an area where the sunscreen had been previously applied. The sunscreen was applied according to the European standard of 2 mg/cm² meaning that after measuring and marking a skin area of 150 cm² and exactly 300 mg of the product were applied. The radiation areas for both UV-B MED-tests were marked with number from 1 to 6 to make the reading of the results easier. The UV-B MED took approximately 3 minutes each and the entirety of the volunteers' bodies (except the radiation areas) were covered with protecting towels, to make sure that that UV-B light would be directed solely to the 6 fields. Between the "UV800k from Waldmann" and the skin of the volunteers a distance of 20 cm was measured and kept during the entire irradiating period.

The UV-A MED consisted of 5 radiation fields, each field was continuously radiated with artificial UV-A light created by the "Dermalight ultra 1 System Dr. Sellmeier" until a specific mJ/cm² dose was reached and messed from the machine itself. After reaching the previously selected dose the irradiation fields would be mechanically closed by the testing supervisor and therefore not receive any more UV-A radiation. The radiation fields and their UV-A dose are listed here:

- 1. Field = 5 J/cm²
- 2. Field = 10 J/cm²
- 3. Field = 15 J/cm²
- 4. Field = 20 J/cm²
- 5. Field = 25 J/cm²

The UV-A MED-tests were carried out on the right dorsal thigh of the volunteers. The first MED were carried out directly on the skin of the participants and the second ones on a previously protected skin area. The same amount of sunscreen (300 mg) was applied on a previously measured skin area (150 cm^2). The radiation areas for both UV-A MED-tests were marked with number from 1 to 5 to make the reading of the results easier. The UV-A MED-tests took approximately 35 minutes and the entirety of the volunteers' bodies (except from the radiation areas) were covered with protecting towels. The machine was positioned at approximately 60 cm to the skin of the volunteers.

All tests took place at the Clinic and Polyclinic for Dermatology and Allergology of the Technical University of Munich

Chromameter

The chromameter is a device that determines the proportion of primary colors in a color mixture. A white light is applied locally. This is reflected from the tissue. The backward scattering light is filtered and the proportion of red, green and blue is measured via a measuring sensor. Each of these colors is measured in its three characteristics (color tone, color saturation, color luminosity) and classified in a three-dimensional model (according to the Commission Internationale de l´Eclairage, CIE).

The Chroma Meter CR-400 from Konica Minolta can scan a color and defining it with the CIELAB color space system by using three different values "L", "a" and "b". "L" is a measurement of lightness from black to white, "a" from green to red and "b" is a from blue to yellow. The exact color of a defined skin area can be determined and quantified. The measurement takes a few seconds. The redness/pigmentation was measured and quantified with the Chromameter for each of the irradiated areas both immediately after and 24 hours after UV-radiation. Each irradiated field was measured three times in order to correct for possible outside interference. Each radiation field received a numeric value and the data was imported and elaborated using IBM SPSS Statistics 26.

Both the UV-A induced redness and the UV-B induced redness were also optically quantified immediately after radiation and 24 hours later and given values ranging from "no redness/pigmentation" to "mild redness/pigmentation" to "strong redness/pigmentation. The chromameter-measured numeric values were compared to the visual cutaneous reaction.

Raster scan opto-acoustic mesoscopy

The Raster Scan Optoacoustic Mesoscopy (RSOM) is a new type of dermatological imaging method that uses the so-called optoacoustic effect. With the help of an ultra-fast pulsed laser, sound waves are induced in the tissue and recorded by an ultrasound detector. Due to its large imaging depth (1,5 mm) and high resolution (5 µm axially, 20 µm laterally), RSOM enables the non-invasive three-dimensional representation of the entirety of the microvascular structures of the skin up to the capillaries for the first time. The method has been used for several years in studies on psoriasis and skin tumors, amongst others. RSOM can directly display and quantify the dose-dependent vascular reaction in UV-induced dermatitis and in high resolution down to the smallest changes in the macroscopic suberythematous area. (Hindelang et al., 2018)

Method

Approximately 24 hours after the MED-tests had been carried out, the biomolecular reactions caused by UV-Light were further investigated. Each of the skin areas, irradiated with UV-B and UV-A, was tested using Raster-Scan-Optoacoustic-Mesoscopy (RSOM) in order to evaluate and quantify the inflammatory response. In figure number 6, the RSOM results on the skin of the volunteers can be seen. It took approximately 90 minutes per participant to fully evaluate the intra- and subcutaneous inflammatory response.

1. UV-B at lowest intensity with sunscreen
2. UV-B at highest intensity with sunscreen
3. UV-B at lowest intensity without sunscreen
4. UV-B at highest intensity without sunscreen
5. UV-A at lowest intensity with sunscreen
6. UV-A at highest intensity with sunscreen
7. UV-A at lowest intensity without sunscreen
8. UV-A at highest intensity without sunscreen

Transepidermal water loss and Stratum Corneum Hydration

SCH

The water content in the stratum corneum has a significant influence on skin appearance. Higher hydration levels correlate with even, soft and elastic skin whereas rough, cracked and flaky skin typically has a lower SCH-value. The water content is also important for the barrier function of the skin, since water makes it difficult for hydrophobic substances to penetrate the skin. Dry skin flakes, often look unattractive, lead to itching and can generally have a negative effect on the quality of life. A disorder in the epidermal cell differentiation process is assumed to be the cause of dry skin and leads to a reduced content of intercellular lipids and moisturizing factors in the stratum corneum. At the same time, sufficient hydration is an essential prerequisite for maintaining the physiological structure and function of the stratum corneum. The most common method for determining stratum corneum hydration is based on measuring the electrical capacity of the skin. This method is based on the linear dependence of the electrical properties of the epidermis on its water content. The dry stratum corneum is a dielectric medium, i.e. only weakly or not at all electrically conductive. When it comes into contact with water, the dielectric properties of the skin change: the higher the water content in the epidermis, the higher the electrical conductivity or capacity. During the capacitive measurement, no current flows through the skin. An electric field is built up instead of using a capacitor, the shape and depth of the electric field depends on the dielectricity of the skin. Since there is no direct contact between the electrodes and the skin during this process, there is no galvanic current flow. The capacitor consists of metallic conductor tracks, which are separated from the skin by a glass layer so that no current flows through the measured object. While a negative charge is built up on one metal track, a positive charge is generated on the other, so that an electric field with mutual attraction builds up between the tracks. During the measurement, the top layer of skin is penetrated by a stray electrical field, the properties of which depend on the water content.

TEWL

One of the roles of the skin is water-loss prevention and immune defense against potentially harmful substances. The stratum corneum, which consists of flat keratinocytes surrounded by special barrier lipids, is primarily responsible for protecting against environmental influences. This intercellular substance is secreted from Odland bodies in the epidermis during the differentiation process of the corneocytes. It protects chemically against potentially harmful substances penetrating from the outside and regulates transepidermal water loss (TEWL). Of particular importance for the barrier lipids are ceramides, free fatty acids and cholesterol. Only when these barrier lipids are present in a physiological relationship to one another can the skin retain its moisture so that its appearance is soft and smooth. In contrast, a disruption of the barrier lipids and the associated increased TEWL or a lack of hydration in the stratum corneum can lead to dry, reddened and irritated skin. The most common method for determining transepidermal water loss is evaporimetry, in which the water evaporation gradient is measured over the surface of the skin. This is done using a hollow cylinder placed vertically on the skin. In the hollow cylinder there are two hygro and temperature sensors at different distances from the skin, from whose data the vapor pressure is calculated.

The difference between the two measuring points allows a direct conclusion on the transepidermal water loss at the examined skin area based on the known gradient. The result is given in grams per hour per square meter (g / h / m²). The basis for this calculation is Fick's law of diffusion, which states that 'the rate of diffusion is proportional to both the surface area and concentration difference and is inversely proportional to the thickness of the membrane'. The particle current density allows a quantitative statement about the directional movement of particles, i.e. how many particles of an object move per unit of time through a unit of area that is perpendicular to the direction of diffusion. (figure 3)

$$\frac{dq}{dt} = -DA\frac{dc}{dx}$$

Figure 3: Fick' law of diffusion: q=quantity of solute

t=membrane surface area; c=concentration; D=diffusion coefficient; dx=membrane thickness; dq/dt=concentration gradient

By measuring the TEWL and STH at day 0, the base values (i.e. the TEWL and STH values prior to sunscreen application) could be compared to the values after respectively 2 and 4 weeks and therefore their change could be measured.

Method

For the TEWL and SCH tests 33 participants were recruited. Every participant received an EGCG cream (approximately 50 ml) and the following instructions on how to apply it:

- The cream needs to be applied at least once a day on the left forearm and on the left side of the face (preferably the left cheek)
- No other skin care products should be applied on the two areas before sunscreen application
- On the test days (day 0, 14, and 28) no creams (not even the sunscreen) should be applied on the test areas

The tests were carried out on day 0, 14 and 28. For the second and third test the participants were allowed to be tested up to 3 days before and after the scheduled testing day to accommodate for personal schedule conflicts, as there was no reason to believe that would lead to any interference in the results.
The room in which the test took place was kept at similar humidity and temperature on each of the testing days, so that the data did not have to be corrected for humidity/temperature changes.
On every testing day the measurements were repeated 3 times for both TEWL and SCH. Only the median of each measurement was considered during the data analysis in order to correct for possible outside interference.
To further investigate the effects of the products 5 participants out of the 33 were selected and in addition to measuring TEWL and SCH values on the "testing side" (left forearm and cheek), the values on the non-testing side (right forearm and cheek) were also measured at day 0,14 and 28 in order to compare the changes in TEWL and STH not only to the same side base value, but also to the natural changes in the skin on the side where no product was applied.
Out of the 33 participants 3 were forced the drop out of the study due to the following reasons:

1. 1 of the participants got pregnant between day 0 and day 14 and pregnancy belonged to the exclusion criteria
2. 1 of the participants was uncompliant and did not apply the cream as instructed
3. 1 of the participants developed a skin rash on the forearm between day 14 and 28, the rash was documented and disappeared quickly after the participant stopped using the cream. It is important

to note that the participant had a previously non-disclosed history of unclear contact dermatitis and that no rash developed on the face despite regular use of the product

All tests took place at the Clinic and Polyclinic for Dermatology and Allergology of the Technical University of Munich

Questionnaires after TEWL and SCH

On the last day of TEWL and SCH measurements the participants were provided with multiple-choice questionnaires. The participants were asked to pick between a range of 4-5 possible answers that would determine their subjective opinion of the EGCG sunscreen.
The time to complete the survey ranged from 10 to 30 minutes per participant.

The questionnaires consisted of 42 questions, 22 of which aimed to collect epidemiological data regarding not only age, sex and occupations but also sun-protection habits and personal sunscreen preferences of the participants. 20 questions aimed to directly evaluate and quantify the volunteer's opinion of the sunscreen. The sunscreens specific questions ranged from overall satisfaction to likelihood to buy the product and subjective feeling of sunscreen protections. In the results some of the most interesting findings with regard to both the "epidemiological" and "EGCG-specific" questions have been listed

All the data was imported and elaborated using IBM SPSS Statistics 26.

Brown-coloring tests

10 participants were recruited at the Clinic and Polyclinic for Dermatology and Allergology of the Technical University of Munich and after appropriate information and written consent, they were provided with an EGCG cream and a white T-shirt. The participants were then asked to apply the cream on their entire upper body including the upper extremities and, immediately afterwards, to put on the white T-shirt and carry out their normal, everyday activities. After approximately 5 hours, the T-shirts were taken off and later collected from the study center to be documented photographically. The brown-colored areas on the T-shirts were compared with a color palette in order to determine and document the exact degree of discoloration and the corresponding intensity. (figure 4)

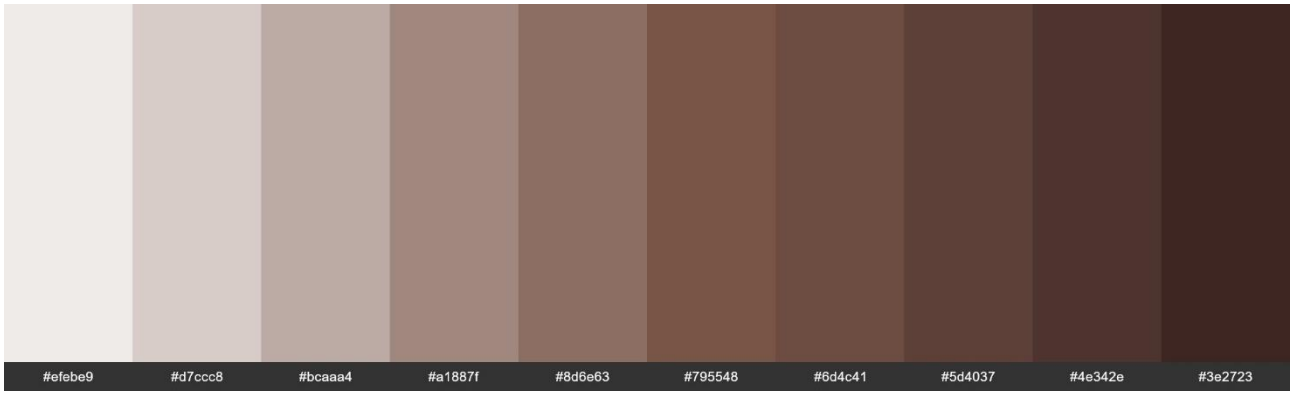


Figure 4: color palette used to quantify the degree of brown coloring.

Sunscreen application dosage

The participants were asked to put on the sunscreen on their upper body "as if they were on the beach on a sunny day", meaning that they would apply as much sunscreen as they are used to do under normal conditions. Using a digital scale, the weight of the cream was measured before and after application. The body surface area of the participants was calculated measuring height and weight, using the DuBois formula and Wallace's rule of nine. (figure 5)

$$\text{DuBois Formula: BSA } (m^2) = \sqrt{\frac{\text{Height (cm)} \times \text{Weight (kg)}}{3{,}600}}$$

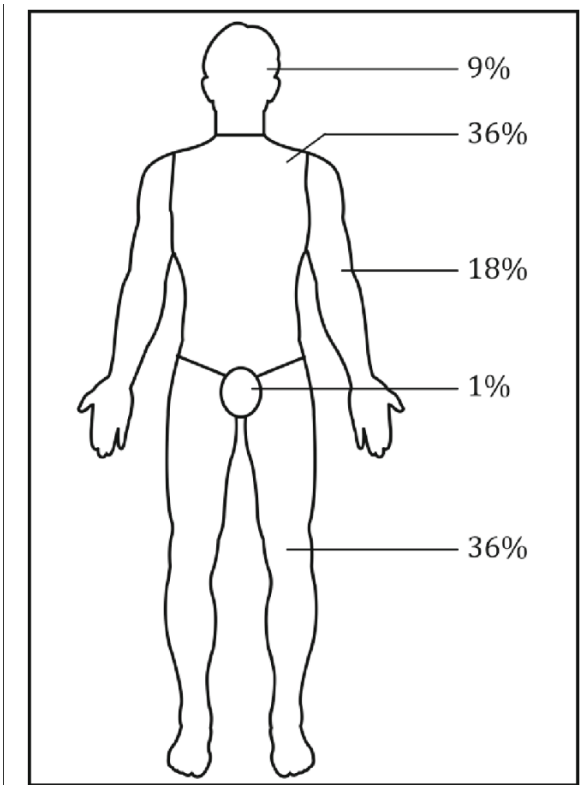


Figure 5: DuBois formula and Wallace's rule of nine Wallace rule of nine

According to Wallace's rule of nine, the upper body and the extremities correspond to approximately 54% of the body surface area, the BSA was therefore multiplicated with 0,54 and to 0,0002 mg/m² to calculate the amount of sunscreen, that the volunteers should have used.

Results

Questionnaires and selection of one sunscreen

Age of the participants in years

N	Valid	104
	Missing	0

Mean	28,96

Table 3 Mean age

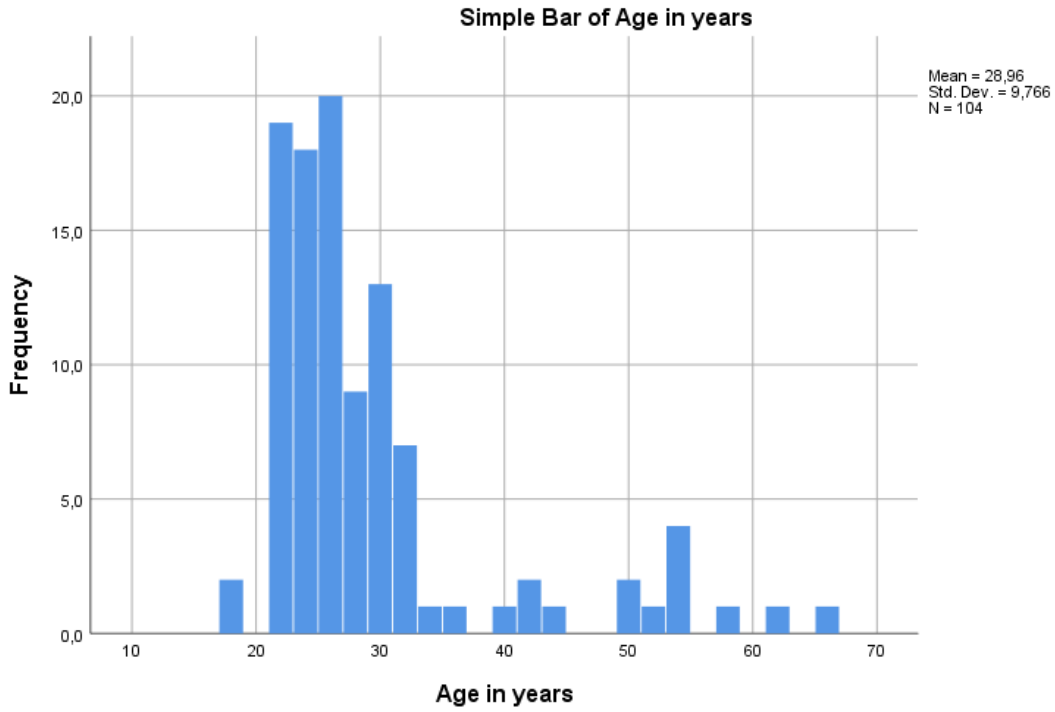


Figure 6 Mean age

The mean age of the participants was approximately 29 years, which means that the data represents better a younger demographic. As can be seen in the bar chart there is a clear spike of participants in the age range 20 to 30.

Sex

		Frequency	Percentage
Valid	Male	38	36,5
	Female	66	63,5
	Total	104	100,0

Table 4 Sex of participants

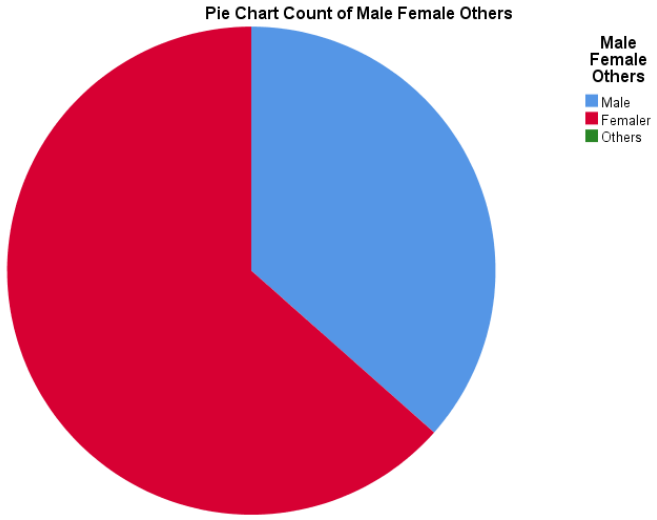


Figure 7 Sex of participants

The epidemiological data showed a clear inclination to a younger and mostly female demographic, which might have skewed the results and not properly considered the opinions of males and older people. It should also be noted that most sunscreen users and buyers are female, and the data might be therefore more representative of the population's buying habits.

How much money do you normally spend on sunscreens?

Answers in euros

N	Valid	96
	Missing	8
Mean		10,8228
Median		10,0000

Table 5 Money participants would spend

When these data were compared with the results of question 31, despite the overall satisfaction with the sunscreens, the participants would still spend less money on them than what they are used to spend.

What is your overall judgment of sunscreen 1-4?

Options:

1. Very good
2. Good
3. Average
4. Bad
5. Very bad

		What is your overall judgment of sunscreen 1?	What is your overall judgment of sunscreen 2?	What is your overall judgment of sunscreen 3?	What is your overall judgment of sunscreen 4?
N	Valid	102	102	102	101
	Missing	2	2	2	3
Mean		2,46	2,23	2,31	2,49

Table 6: Overall judgment

Mean evaluation of sunscreen 1-4

1. 2,46
2. 2,23 → Best sunscreen evaluation
3. 2,31
4. 2,49

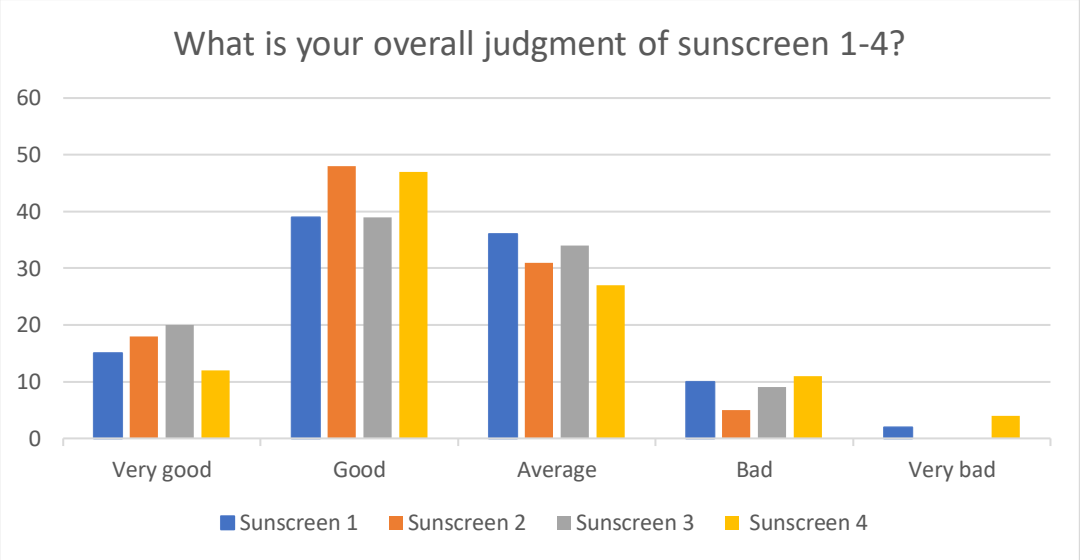


Figure 8: Overall judgment

The sunscreen number 2 reached a mean of 2,23 making it closer to the highest value (1 = very good) and was therefore the cream with the best overall score in this subsection of the questionnaire.

How convinced are you of sunscreen 1-4 as a sun protection product?

Options:

1. Very convinced
2. Mostly convinced
3. Neither nor
4. Mostly not convinced
5. Not convinced at all

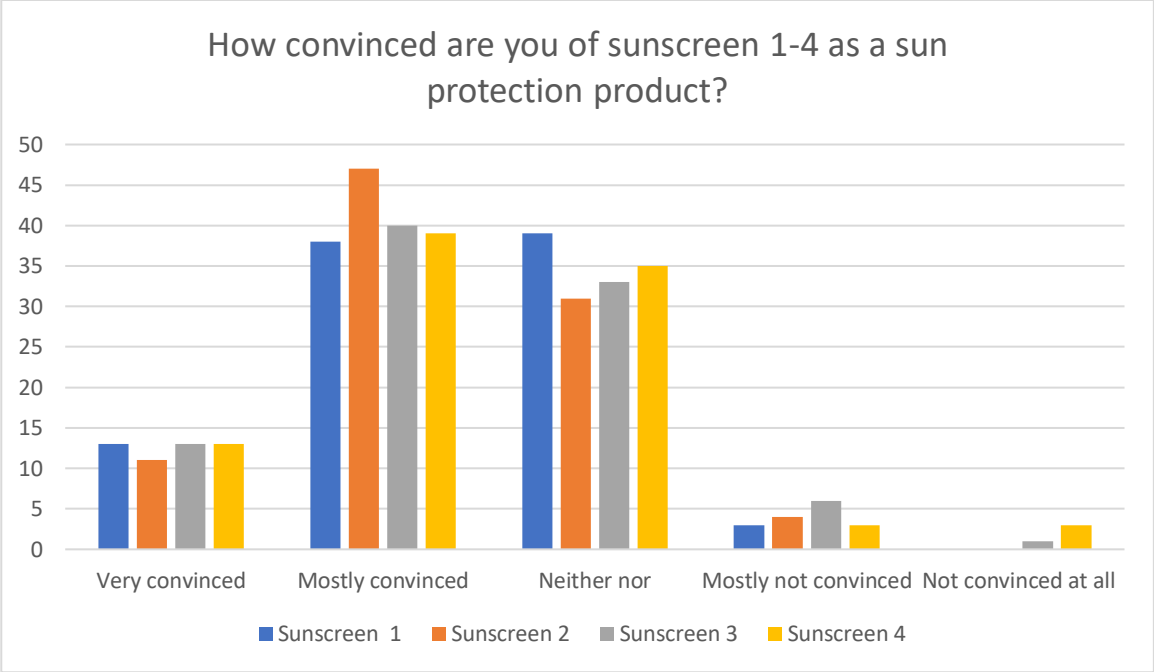
		How convinced are you of sunscreen 1 as a sun protection product?	How convinced are you of sunscreen 2 as a sun protection product?	How convinced are you of sunscreen 3 as a sun protection product?	How convinced are you of sunscreen 4 as a sun protection product?
N	Valid	93	93	93	93
	Missing	11	11	11	11
Mean		2,34	2,30	2,38	2,40

Table 7 Convincement level

Mean evaluation of sunscreen 1-4

1. 2,34
2. 2,30 → Best sunscreen evaluation
3. 2,38
4. 2,40

Figure 9: Convincement level



The sunscreen number 2 reached a mean of 2,30 making it closer to the highest value (1 = very convinced) and was therefore the sunscreen which convinced the participants the most out of the 4.

Would you recommend sunscreen 1-4 to a friend?

Question number 28 of the questionnaire

Options:

1. Very likely
2. Likely
3. Unlikely

4. Very unlikely

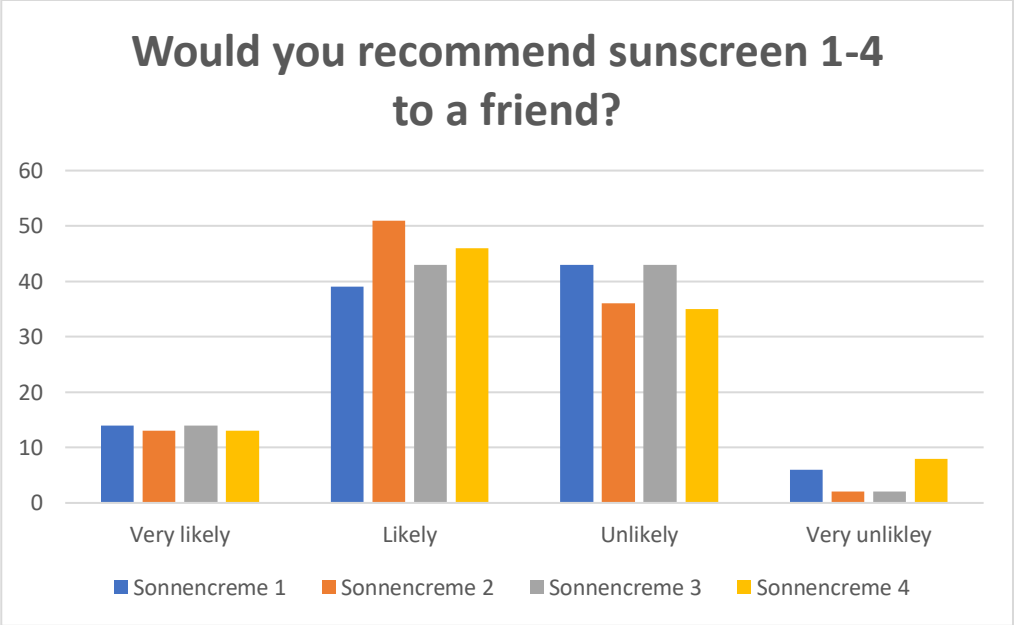
		Would you recommend sunscreen 1 to a friend?	Would you recommend sunscreen 2 to a friend?	Would you recommend sunscreen 3 to a friend?	Would you recommend sunscreen 4 to a friend?
N	Valid	102	102	102	102
	Missing	2	2	2	2
Mean		2,40	2,26	2,32	2,37

Table 8 Recommendation to a friend

Mean evaluation of sunscreen 1-4

1. 2,40
2. 2,26 → Best sunscreen evaluation
3. 2,32
4. 2,37

Figure 10: Recommendation to a friend



The sunscreen number 2 reached a mean of 2,26 making it closer to the highest value (1 = very likely) and was therefore the sunscreen which would be recommended by more participants

Would you buy sunscreen 1-4?

Question number 30 of the questionnaire

Sunscreen 1

		Frequency	Percentage	Valid percentages
Valid	Yes	39	37,5	38,2

	No	63	60,6	61,8
	Total	102	98,1	100,0
Missing	System	2	1,9	
Total			100,0	

Table 9 Buying tendencies sunscreen 1

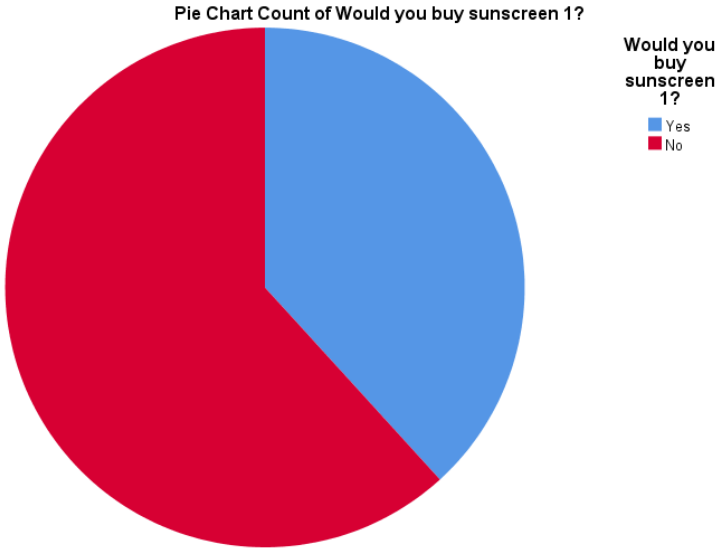


Figure 11 Buying tendencies sunscreen 1

Sunscreen 2 → Best sunscreen evaluation

		Frequency	Percentage	Valid percentages
Valid	Yes	55	52,9	53,9
	No	47	45,2	46,1
	Total	102	98,1	100,0
Missing	System	2	1,9	
Total			100,0	

Table 10 Buying tendencies sunscreen 2

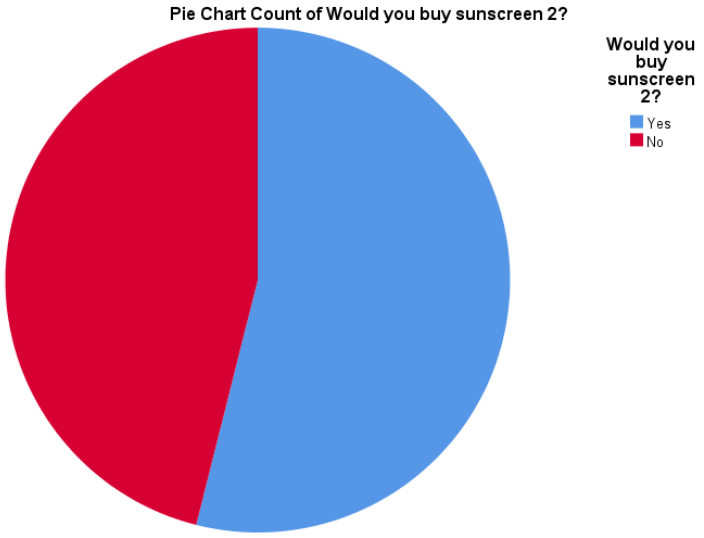


Figure 12 Buying tendencies sunscreen 2

Sunscreen 3

		Frequency	Percentage	Valid percentages
Valid	Yes	43	41,3	42,2
	No	59	56,7	57,8
	Total	102	98,1	100,0
Missing	System	2	1,9	
Total			100,0	

Table 11 Buying tendencies sunscreen 3

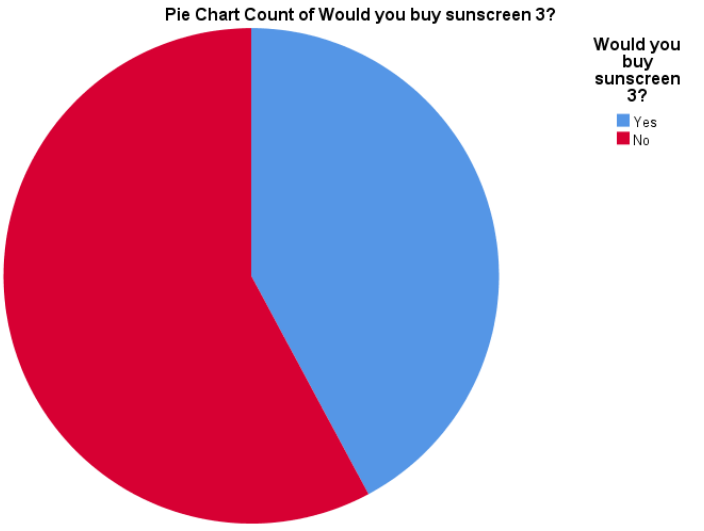


Figure 13 Buying tendencies sunscreen 3

Sunscreen 4

		Frequency	Percentage	Valid percentages
Valid	Yes	45	43,3	45,9
	No	53	51,0	54,1
	Total	98	94,2	100,0
Missing	System	6	5,8	
Total		104	100,0	

Table 12 Buying tendencies sunscreen 4

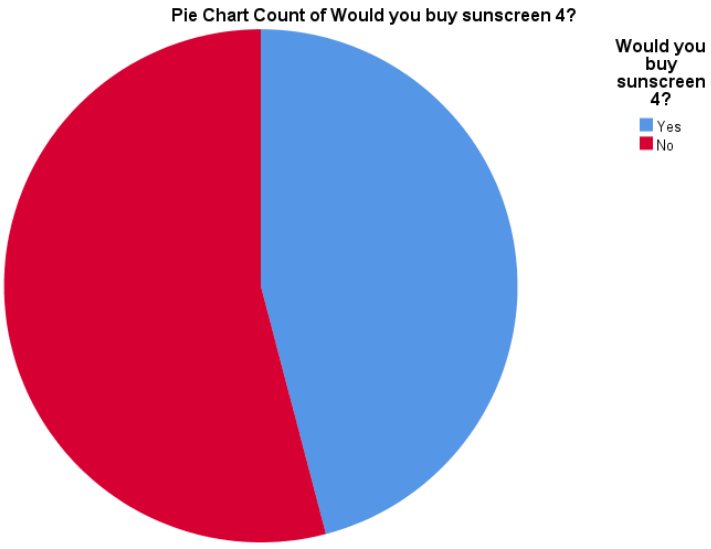


Figure 14 Buying tendencies sunscreen 4

The second sunscreen was the only one to score more than 50% in this subsection of the questionnaire, these results are interesting for different reasons:

1. Sunscreens 2 and 3 have had similar results to all the other questions, but, when confronted with the possibility of buying one of the tested products the participants clearly indicated the preference for the cream that looked whiter and did not have the typical EGCG brown color
2. The answer regarding the satisfaction and the overall judgement for the products were positive for all the 4 creams, but only the second was able to reach values over 50% and would therefore be bought by more than half of the participants

How much would you spend for sunscreen 1-4?

Question number 31 f the questionnaire

Answers in Euros

		How much would you spend for cream 1?	How much would you spend for cream 2?	How much would you spend for cream 3?	How much would you spend for cream 4?
N	Valid	76	87	83	83

Missing	28	17	21	21
Mean	8,7499	8,9252	9,4577	9,8553
Median	8,5000	9,0000	10,0000	10,0000

Table 13 Money participants would spend

Mean of sunscreen 1-4

1. 8,5 Euro
2. 8,9 Euro
3. 9,5 Euro
4. 9,9 Euro → Highest paying price

The results to this question show that, despite averaging worse in all the other questions, and not reaching 50% in the "would you buy this product" subsection, sunscreen 4 still outscores the other 3

MED and chromameter

UV-B

Colorimeter measurement

The chromameter values were compared for each of the 6 irradiated fields for both protected and unprotected skins.

UV-B Chromameter

		Mean	N	Std. Deviation	Std. Error Mean
Field 1	Sunscreen	6,8100	10	2,04171	,64565
	No Sunscreen	7,1200	10	1,84021	,58192
Field 2	Sunscreen	6,4080	10	1,96061	,62000
	No Sunscreen	7,6640	10	1,98571	,62794
Field 3	Sunscreen	6,3270	10	1,28246	,40555
	No Sunscreen	8,7610	10	2,99339	,94659
Field 4	Sunscreen	6,7740	10	2,76682	,87495
	No Sunscreen	10,4300	10	3,66411	1,15869
Field 5	Sunscreen	6,8740	10	1,72391	,54515
	No Sunscreen	11,6360	10	3,48871	1,10323
Field 6	Sunscreen	7,2210	10	1,33512	,42220
	No Sunscreen	12,9000	10	3,01279	,95273

Table 14 UVB Chromameter

Paired differences between protected and unprotected fields

	Paired Differences	t	df	Sig. (2-tailed)

		Mean	Std. Deviation	Std. Error Mean	95% Confidence Interval of the Difference Lower	Upper			
Field 1	Sunscreen – No Sunscreen	-,31000	,74134	,23443	-,84032	,22032	-1,322	9	,219
Field 2	Sunscreen – No Sunscreen	-1,25600	1,35419	,42823	-2,22473	-,28727	-2,933	9	,017
Field 3	Sunscreen – No Sunscreen	-2,43400	2,88727	,91303	-4,49943	-,36857	-2,666	9	,026
Field 4	Sunscreen – No Sunscreen	-3,65600	2,56613	,81148	-5,49170	-1,82030	-4,505	9	,001
Field 5	Sunscreen – No Sunscreen	-4,76200	3,33825	1,05565	-7,15004	-2,37396	-4,511	9	,001
Field 6	Sunscreen – No Sunscreen	-5,67900	2,90812	,91963	-7,75935	-3,59865	-6,175	9	,000

Table 15 UVB paired differences protected/unprotected

Looking at the results of the UV-B MED-tests for both the protected and unprotected fields, the means are consistently lower in the protected side, which indicates a lower level of redness of the skin. The skin which had previously been protected with the EGCG sunscreen showed lower erythema values. These results are consistent with our in vitro test and with the existing literature with regard to EGCG and green tea extracts in general.

Furthermore, it should be noted that all pairings but the first one (25 mJ/cm²) have a p-Values lower than 0,05 and are therefore significant. It is also interesting to notice that the means difference gets wider with higher UV-B intensities, which seems to show that the sunscreen does a consistent job of protecting the skin despite the raising intensity of the UV-B light

Visible reaction measurement

The visible reaction values for each of the 6 irradiated fields for both protected and unprotected skin fields were compared 24 hours after radiation. The data for the visible reaction directly after UV-B radiation were also collected, but not shown as there were no interesting results. It takes approximately 24 hours to fully see and be able to evaluate the results of UV-B radiation because of the nature of the radiation itself. Directly after UV-B radiation the skin had not had enough time to react to the radiation and it was not possible to evaluate any reaction.

Values:

- 0 = no reaction
- 1 = mild reaction
- 2 = strong reaction

UV-B visible reaction 24h post exposure

	Mean	N	Std. Deviation	Std. Error Mean

Field 1	Sunscreen	,00[a]	10	,000	,000
	No Sunscreen	,00[a]	10	,000	,000
Field 2	Sunscreen	,00	10	,000	,000
	No Sunscreen	,30	10	,483	,153
Field 3	Sunscreen	,00	10	,000	,000
	No Sunscreen	,70	10	,823	,260
Field 4	Sunscreen	,10	10	,316	,100
	No Sunscreen	1,10	10	,738	,233
Field 5	Sunscreen	,10	10	,316	,100
	No Sunscreen	1,50	10	,707	,224
Field 6	Sunscreen	,10	10	,316	,100
	No Sunscreen	1,50	10	,707	,224

Table 16 UVB reaction after 24h

Paired differences in UV-B visible reaction 24h post exposure

		Paired Differences					t	df	Sig. (2-tailed)
		Mean	Std. Deviation	Std. Error Mean	95% Confidence Interval of the Difference Lower	Upper			
Field 2	Sunscreen – No Sunscreen	-,300	,483	,153	-,646	,046	-1,964	9	,081
Field 3	Sunscreen – No Sunscreen	-,700	,823	,260	-1,289	-,111	-2,689	9	,025
Field 4	Sunscreen – No Sunscreen	-1,000	,667	,211	-1,477	-,523	-4,743	9	,001
Field 5	Sunscreen – No Sunscreen	-1,400	,699	,221	-1,900	-,900	-6,332	9	,000
Field 6	Sunscreen – No Sunscreen	-1,400	,699	,221	-1,900	-,900	-6,332	9	,000

Table 17 UVB reaction after 24h paired differences

At the 6th pairing, meaning at an irradiation of 150 mJ/cm², the mean for the unprotected side reaches 1,5 whereas the protected side stays at 0,10. Knowing that 2,0 corresponds to “strong reaction” and 0 to “no reaction”, the protected side consistently shows little to no erythema. It should also be noted that from the 3rd paring on, all the mean differences have a p-value lower than 0,05 and are therefore significant. The means differences also get progressively wider starting from the second and reaching the 6th pairing, which

once more are consistent with the chromometer findings and show a consistent UV-B protection at high UV-B radiation intensity. These results are consistent with the chromameter measured findings.

UV-A

Colorimeter measurement

The chromameter values for each of the 5 irradiated fields for both protected and unprotected skins were compared.

UV-A Chromameter

		Mean	N	Std. Deviation	Std. Error Mean
Field 1	Sunscreen	6,0250	10	1,77026	,55981
	No Sunscreen	6,4850	10	1,55803	,49269
Field 2	Sunscreen	5,6180	10	1,25860	,39800
	No Sunscreen	6,8020	10	1,79486	,56758
Field 3	Sunscreen	5,7180	10	1,21155	,38313
	No Sunscreen	6,6620	10	1,44871	,45812
Field 4	Sunscreen	6,2910	10	1,09924	,34761
	No Sunscreen	6,9440	10	1,48865	,47075
Field 5	Sunscreen	6,6790	10	1,30554	,41285
	No Sunscreen	7,5320	10	1,46248	,46248

Table 18 UVA Chromameter

Paired differences between protected and unprotected fields

		Paired Differences					t	df	Sig. (2-tailed)
		Mean	Std. Deviation	Std. Error Mean	95% Confidence Interval of the Difference Lower	Upper			
Field 1	Sunscreen – No Sunscreen	-,46000	,95371	,30159	-1,14224	,22224	-1,525	9	,162
Field 2	Sunscreen – No Sunscreen	-1,18400	1,64588	,52047	-2,36139	-,00661	-2,275	9	,049
Field 3	Sunscreen – No Sunscreen	-,94400	1,29254	,40874	-1,86863	-,01937	-2,310	9	,046
Field 4	Sunscreen – No Sunscreen	-,65300	,75497	,23874	-1,19307	-,11293	-2,735	9	,023
Field 5	Sunscreen – No Sunscreen	-,85300	,87328	,27616	-1,47771	-,22829	-3,089	9	,013

Table 19 UVA paired differences protected/unprotected

The UV-A results are consistent with the UV-B findings. The means for the protected field are lower than for the unprotected. All but the first pairing (5 J/cm²) have a p-value lower than 0,05 and are therefore significant. It is interesting to notice that the means differences are this time wider for the 3rd and second pairing rather than for the 5th one. It should also be mentioned the standard deviation is also higher for said pairings which might explain the apparent inconsistency.

Visible reaction measurement

The visible reaction values for each of the 5 irradiated fields for both protected and unprotected skin fields were compared directly and 24 hours after radiation. As typical for UV-A light, a very intense pigmentation is visible directly after exposure and slowly decreases in intensity reaching a relative plateau approximately 24h after irradiation, that is why the reactions were measured both at hour 0 and 24 after exposure. (Gerd Plewig, 2018)

Values:

- 0 = no reaction (redness/pigmentation)
- 1 = mild reaction (redness/pigmentation)
- 2 = strong reaction (redness/pigmentation)

UV-A visible reaction 24h post exposure

		Mean	N	Std. Deviation	Std. Error Mean
Field 1	Sunscreen	,00[a]	10	,000	,000
	No Sunscreen	,00[a]	10	,000	,000
Field 2	Sunscreen	,00	10	,000	,000
	No Sunscreen	,20	10	,422	,133
Field 3	Sunscreen	,00	10	,000	,000
	No Sunscreen	,70	10	,823	,260
Field 4	Sunscreen	,00	10	,000	,000
	No Sunscreen	1,10	10	,738	,233
Field 5	Sunscreen	,20	10	,422	,133
	No Sunscreen	1,30	10	,675	,213

Table 20 UVB reaction after 24h

Paired differences in UV-A visible reaction 24h post exposure

		Paired Differences					t	df	Sig. (2-tailed)
					95% Confidence Interval of the Difference				
		Mean	Std. Deviation	Std. Error Mean	Lower	Upper			
Field 2	Sunscreen – No Sunscreen	-,200	,422	,133	-,502	,102	-1,500	9	,168
Field 3	Sunscreen – No Sunscreen	-,700	,823	,260	-1,289	-,111	-2,689	9	,025

Field 4	Sunscreen – No Sunscreen	-1,100	,738	,233	-1,628	-,572	-4,714	9	,001
Field 5	Sunscreen – No Sunscreen	-1,100	,568	,180	-1,506	-,694	-6,128	9	,000

Table 21 UVB reaction after 24h paired differences

At the 5th pairing, meaning at an irradiation of 25 J/cm2, the mean for the unprotected side reaches 1,3 whereas the protected side stays at 0,20. Knowing that 2,0 corresponds to "strong reaction" and 0 to "no reaction", the protected side consistently shows little to no pigmentation. It should also be noted that from the 3rd paring on all the mean differences have a p-value lower than 0,05 and are therefore significant.

The means differences also get progressively wider starting from the second and reaching the 5th pairing which once more are consistent with the colorimeter findings and show a consistent UV-A protection at high UV-A radiation intensity. These findings are consistent with the UV-B results.

UV-A visible reaction at 0h and 24h:

The visible reaction on each side (protected and unprotected) after 24h were compared with their value directly after irradiation.

UV-A visible reaction at hour 0 and 24 post exposure on sunscreen side

		Mean	N	Std. Deviation	Std. Error Mean
Field 1	Sunscreen 0h	,00[a]	10	,000	,000
	Sunscreen 24h	,00[a]	10	,000	,000
Field 2	Sunscreen 0h	,00[a]	10	,000	,000
	Sunscreen 24h	,00[a]	10	,000	,000
Field 3	Sunscreen 0h	,10	10	,316	,100
	Sunscreen 24h	,00	10	,000	,000
Field 4	Sunscreen 0h	,60	10	,699	,221
	Sunscreen 24h	,00	10	,000	,000
Field 5	Sunscreen 0h	1,00	10	,943	,298
	Sunscreen 24h	,20	10	,422	,133

Table 22 UV-A visible reaction at hour 0 and 24 post exposure on sunscreen side

Paired differences in UV-A visible reaction at hour 0 and 24 post exposure on the unprotected (no sunscreen) side

		Mean	N	Std. Deviation	Std. Error Mean
Field 1	No Sunscreen 0h	,00[a]	10	,000	,000
	No Sunscreen 24h	,00[a]	10	,000	,000
Field 2	No Sunscreen 0h	,50	10	,527	,167
	No Sunscreen 24h	,20	10	,422	,133
Field 3	No Sunscreen 0h	1,30	10	,675	,213
	No Sunscreen 24h	,70	10	,823	,260

Field 4	No Sunscreen 0h	1,80	10	,632	,200
	No Sunscreen 24h	1,10	10	,738	,233
Field 5	No Sunscreen 0h	1,80	10	,632	,200
	No Sunscreen 24h	1,30	10	,675	,213

Table 23 Paired differences in UV-A visible reaction at hour 0 and 24 post exposure

Comparing the visible reaction at hour 0 and 24, the results are consistent with the existing literature, which shows an initial very intense pigmentation which gradually decreases over 24 hours.

The results are also consistent with the previous test showing that on the unprotected side the pigmentation is stronger at both hour 0 and 24, when compared to the protected side. Only on the 5th and last field there is a reaction for both the protected and the unprotected side after 24 hours. The reaction of the protected side shows a much lower intensity (0,20 vs 1,30). The reason for this difference was speculated to rely not only on the protective effect of the sunscreen, but also on the anti-inflammatory qualities of the EGCG molecule itself which might have lowered the intensity of the pigmentation over the 24 hours window.

Raster scan opto-acoustic mesoscopy

As can be seen in figures 15-16, it was not possible to optically identify a difference in inflammation and vascular reaction between protected and unprotected skin after UV-A or UV-B radiation. Both images show a similar vascular inflammatory response despite the difference in UV light intensity they had been exposed to.

Since no significant differences were shown in the inflammatory response to the radiation, the RSOM-Test proved to be unsuitable for the tests.

Figure 15: RSOM preparation and implementation

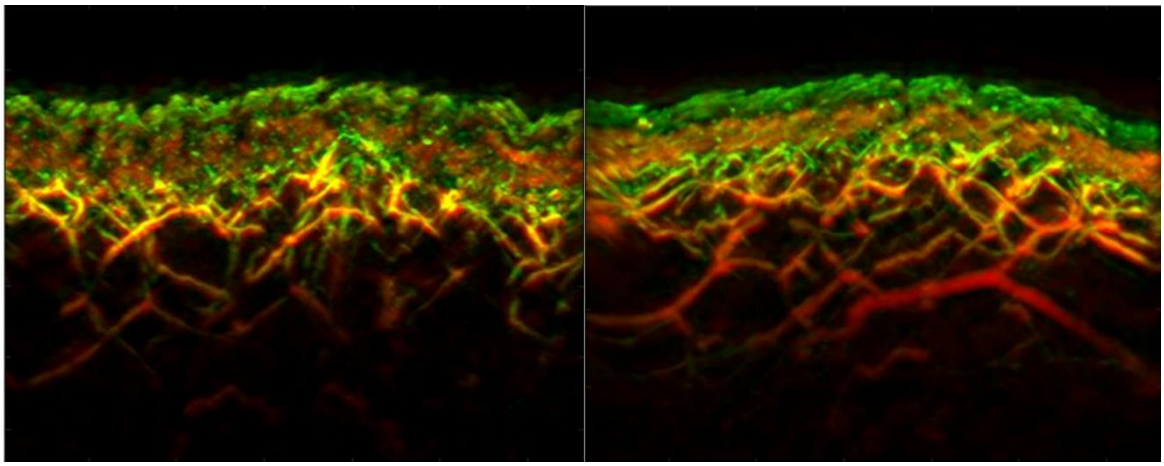


Figure 16: RSOM Test after UV-A radiation on both unprotected and protected skin

Transepidermal water loss and Stratum Corneum Hydration

To analyze the results paired sample t-tests in IBM SPSS Statistics 26 were used.

Stratum Corneum Hydration

SCH Day 0 to day 14 (Arm)

		Mean	N	Std. Deviation	Std. Error Mean
SCH Arm	Day 0	34,8063	32	9,63471	1,70319
	Day 14	26,4094	32	12,16246	2,15004

Table 24 Stratum Corneum Hydration day 0 to 14 (arm)

		Paired Differences					t	df	Sig. (2-tailed)
		Mean	Std. Deviation	Std. Error Mean	95% Confidence Interval of the Difference Lower	Upper			
SCH Arm	Day 0 to 14	8,39687	11,70061	2,06839	4,17836	12,61539	4,060	31	,000

Table 25 Paired differences in Stratum Corneum Hydration day 0 to 14 (arm)

SCH Day 0 to day 14 (Face)

		Mean	N	Std. Deviation	Std. Error Mean
SCH Face	Day 0	39,5188	32	15,38754	2,72016
	Day 14	29,8888	32	14,45223	2,55482

Table 26 Stratum Corneum Hydration day 0 to 14 (face)

		Paired Differences					t	df	Sig. (2-tailed)
		Mean	Std. Deviation	Std. Error Mean	95% Confidence Interval of the Difference Lower	Upper			
SCH Face	Day 0 to 14	9,63000	17,87056	3,15910	3,18697	16,07303	3,048	31	,005

Table 27 Paired differences in Stratum Corneum Hydration day 0 to 14 (face)

SCH Day 14 to day 28 (Arm)

		Mean	N	Std. Deviation	Std. Error Mean
SCH Arm	Day 14	26,0194	31	12,15838	2,18371
	Day 28	23,4581	31	8,91328	1,60087

Table 28 Stratum Corneum Hydration day 14 to 28 (arm)

		Paired Differences					t	df	Sig. (2-tailed)
		Mean	Std. Deviation	Std. Error Mean	95% Confidence Interval of the Difference Lower	Upper			
SCH Arm	Day 14 to 28	2,56129	8,22649	1,47752	-,45621	5,57879	1,734	30	,093

Table 29 Paired differences in Stratum Corneum Hydration day 14 to 28 (arm)

SCH Day 14 to day 28 (Face)

		Mean	N	Std. Deviation	Std. Error Mean
SCH Face	Day 14	30,1658	31	14,60449	2,62304
	Day 28	30,2052	31	17,73251	3,18485

Table 30 Stratum Corneum Hydration day 14 to 28 (face)

		Paired Differences					t	df	Sig. (2-tailed)
		Mean	Std. Deviation	Std. Error Mean	95% Confidence Interval of the Difference Lower	Upper			
SCH Face	Day 14 to 28	-,03935	11,52056	2,06915	-4,26513	4,18642	-,019	30	,985

Table 31 Paired differences in Stratum Corneum Hydration day 14 to 28 (Face)

SCH Day 0 to day 28 (Arm)

		Mean	N	Std. Deviation	Std. Error Mean

SCH Arm	Day 0	35,0839	31	9,66299	1,73552
	Day 28	23,4581	31	8,91328	1,60087

Table 32 Stratum Corneum Hydration day 0 to 28 (arm)

		Paired Differences							
					95% Confidence Interval of the Difference				
		Mean	Std. Deviation	Std. Error Mean	Lower	Upper	t	df	Sig. (2-tailed)
SCH Arm	Day 0 to 28	11,62581	9,75124	1,75137	8,04902	15,20259	6,638	30	,000

Table 33 Paired differences in Stratum Corneum Hydration day 0 to 28 (arm)

SCH Day 0 to day 28 (Face)

		Mean	N	Std. Deviation	Std. Error Mean
SCH Face	Day 0	39,6161	31	15,63187	2,80757
	Day 28	30,2052	31	17,73251	3,18485

Table 34 Stratum Corneum Hydration day 0 to 28 (face)

		Paired Differences							
					95% Confidence Interval of the Difference				
		Mean	Std. Deviation	Std. Error Mean	Lower	Upper	t	df	Sig. (2-tailed)
SCH Face	Day 0 to 28	9,41097	18,94935	3,40340	2,46029	16,36165	2,765	30	,010

Table 35 Paired differences in Stratum Corneum Hydration day 0 to 28 (face)

Transepidermal Water Loss

TEWL Day 0 to 14 (arm)

		Mean	N	Std. Deviation	Std. Error Mean
TEWL Arm	Day 0	11,7803	32	4,49248	,79417
	Day 14	19,1019	32	11,43399	2,02126

Table 36 Transepidermal water loss day 0 to 14 (arm)

	Paired Differences						
				95% Confidence Interval of the Difference			
Mean	Std. Deviation	Std. Error Mean			t	df	Sig. (2-tailed)

					Lower	Upper			
TEWL Arm	Day 0 to 14	-7,32156	12,84328	2,27039	-11,95206	-2,69107	-3,225	31	,003

Table 37 Paired differences in Transepidermal water loss day 0 to 14 (arm)

TEWL Day 0 to 14 (face)

		Mean	N	Std. Deviation	Std. Error Mean
TEWL Face	Day 0	19,1506	32	10,34500	1,82875
	Day 14	23,6169	32	6,08291	1,07532

Table 38 Transepidermal water loss day 0 to 14 (face)

		Paired Differences							
					95% Confidence Interval of the Difference				
		Mean	Std. Deviation	Std. Error Mean	Lower	Upper	t	df	Sig. (2-tailed)
TEWL Face	Day 0 to 14	-4,46625	10,09845	1,78517	-8,10713	-,82537	-2,502	31	,018

Table 39 Paired differences in Transepidermal water loss day 0 to 14 (face)

TEWL Day 14 to 28 (arm)

		Mean	N	Std. Deviation	Std. Error Mean
TEWL Arm	Day 14	19,2161	31	11,60441	2,08421
	Day 28	18,8981	31	8,43404	1,51480

Table 40 Transepidermal water loss day 14 to 28 (arm)

		Paired Differences							
					95% Confidence Interval of the Difference				
		Mean	Std. Deviation	Std. Error Mean	Lower	Upper	t	df	Sig. (2-tailed)
TEWL Arm	Day 14 to 28	,31806	12,07689	2,16907	-4,11177	4,74790	,147	30	,884

Table 41 Transepidermal water loss day 14 to 28 (arm)

TEWL Day 14 to 28 (face)

		Mean	N	Std. Deviation	Std. Error Mean
TEWL Face	Day 14	23,5081	31	6,15172	1,10488
	Day 28	23,5968	31	7,51945	1,35053

Table 42 Transepidermal water loss day 14 to 28 (face)

		Paired Differences					t	df	Sig. (2-tailed)
		Mean	Std. Deviation	Std. Error Mean	95% Confidence Interval of the Difference Lower	Upper			
TEWL Face	Day 14 to 28	-,08871	7,27556	1,30673	-2,75741	2,57999	-,068	30	,946

Table 43 Paired differences in Transepidermal water loss day 14 to 28 (face)

TEWL Day 0 to 28 (arm)

		Mean	N	Std. Deviation	Std. Error Mean
TEWL Arm	Day 0	11,5829	31	4,42341	,79447
	Day 28	18,8981	31	8,43404	1,51480

Table 44 Transepidermal water loss day 0 to 28 (arm)

		Paired Differences					t	df	Sig. (2-tailed)
		Mean	Std. Deviation	Std. Error Mean	95% Confidence Interval of the Difference Lower	Upper			
TEWL Arm	Day 0 to 28	-7,31516	8,99285	1,61516	-10,61377	-4,01656	-4,529	30	,000

Table 45 Paired differences in Transepidermal water loss day 0 to 28 (arm)

TEWL Day 0 to 28 (face)

		Mean	N	Std. Deviation	Std. Error Mean
TEWL Face	Day 0	19,1077	31	10,51311	1,88821
	Day 28	23,5968	31	7,51945	1,35053

Table 46 Paired differences in Transepidermal water loss day 0 to 28 (arm)

		Paired Differences					t	df	Sig. (2-tailed)
		Mean	Std. Deviation	Std. Error Mean	95% Confidence Interval of the Difference Lower	Upper			
TEWL Face	Day 0 to 28	-4,48903	12,98276	2,33177	-9,25115	,27308	-1,925	30	,064

Table 47 Paired differences in Transepidermal water loss day 0 to 28 (face)

Results

- From day 0 to day 14 there is a significant reduction in SCH levels in the arms of the participants, from 34,8 to 26,4
- From day 0 to day 14 there is a significant reduction in SCH levels in the cheeks of the participants, from 39,5 to 29,9
- From day 14 to day 28 there is no significant change in SCH levels in the arms of the participants ($p > 0,05$)
- From day 14 to day 28 there is no significant change in SCH levels in the cheeks of the participants ($p > 0,05$)
- From day 0 to day 28 there is a significant reduction in SCH levels in the arms of the participants, from 35,1 to 23,5
- From day 0 to day 28 there is a significant reduction in SCH levels in the cheeks of the participants, from 39,6 to 30,2
- From day 0 to day 14 there is a significant increase in TEWL levels in the arms of the participants, from 11,8 to 19,1
- From day 0 to day 14 there is a significant increase in TEWL levels in the cheeks of the participants, from 19,1 to 23,6
- From day 14 to day 28 there is no significant increase in TEWL levels in the arms of the participants ($p > 0,05$)
- From day 14 to day 28 there is no significant increase in TEWL levels in the cheeks of the participants ($p > 0,05$)
- From day 0 to day 28 there is a significant increase in TEWL levels in the arms of the participants, from 11,6 to 18,9
- From day 0 to day 28 there is no significant increase in TEWL levels in the cheeks of the participants ($p > 0,05$)

Left-right test

		Mean	N	Std. Deviation	Std. Error Mean
SCH day 14	Left (sunscreen)	16,7000	5	4,27142	1,91024
	Right	25,2000	5	10,19338	4,55862
TEWL day 14	Left (sunscreen)	15,6360	5	6,17652	2,76222
	Right	12,1340	5	2,10431	,94108
SCH day 28	Left (sunscreen)	17,8600	5	4,93640	2,20762
	Right	26,5400	5	6,15167	2,75111
	Left (sunscreen)	16,6820	5	4,77686	2,13628

TEWL day 28	Right	13,3440	5	2,36530	1,05779

Table 48 Left-right Test

		Paired Differences					t	df	Sig. (2-tailed)
		Mean	Std. Deviation	Std. Error Mean	95% Confidence Interval of the Difference Lower	Upper			
SCH day 14	Left (sunscreen) – right	-8,50000	6,32535	2,82878	-16,35395	-,64605	-3,005	4	,040
TEWL day 14	Left (sunscreen) – right	3,50200	4,62999	2,07060	-2,24689	9,25089	1,691	4	,166
SCH day 28	Left (sunscreen) – right	-8,68000	9,65955	4,31988	-20,67392	3,31392	-2,009	4	,115
TEWL day 28	Left (sunscreen) – right	3,33800	2,71304	1,21331	-,03068	6,70668	2,751	4	,051

Table 49 Left-right Test paired samples

As previously mentioned under the "objective" subsection 5 out of 33 candidates were selected and TEWL and SCH measurements were carried out on both the left arm (where the sunscreen has been daily applied) and the right arm (where no sunscreen was applied at any time during the tests). The measurements took place on day 0, 14 and 28 exactly like for the other participants, the 5 people had also been instructed, exactly like the others, to not apply any products on the testing areas on the measurement days.

The aim of this test was to compare the change in TEWL and SCH values over time not only with their base value, but also with physiological changes in the skin of the participants over the 4 weeks periods. This would make it possible to see if the changes on the sunscreen side were caused by the product itself or if they were just normal "physiological" TEWL and SCH changes.

Over the 4 weeks periods there is no significant improvement in both the TEWL and SCH values on both sides. This confirms the findings that showed that the EGCG-sunscreen was not able to improve the hydration and water-loss levels.

Questionnaires after TEWL and SCH

Some of the more interesting results of the questionnaire are listed below. To analyze the results, IBM SPSS Statistics 26 was used.

Age in years

	N	Minimum	Maximum	Mean	Std. Deviation
Age in years	30	22	50	27,70	5,772
Valid N (listwise)	30				

Table 50 Mean age

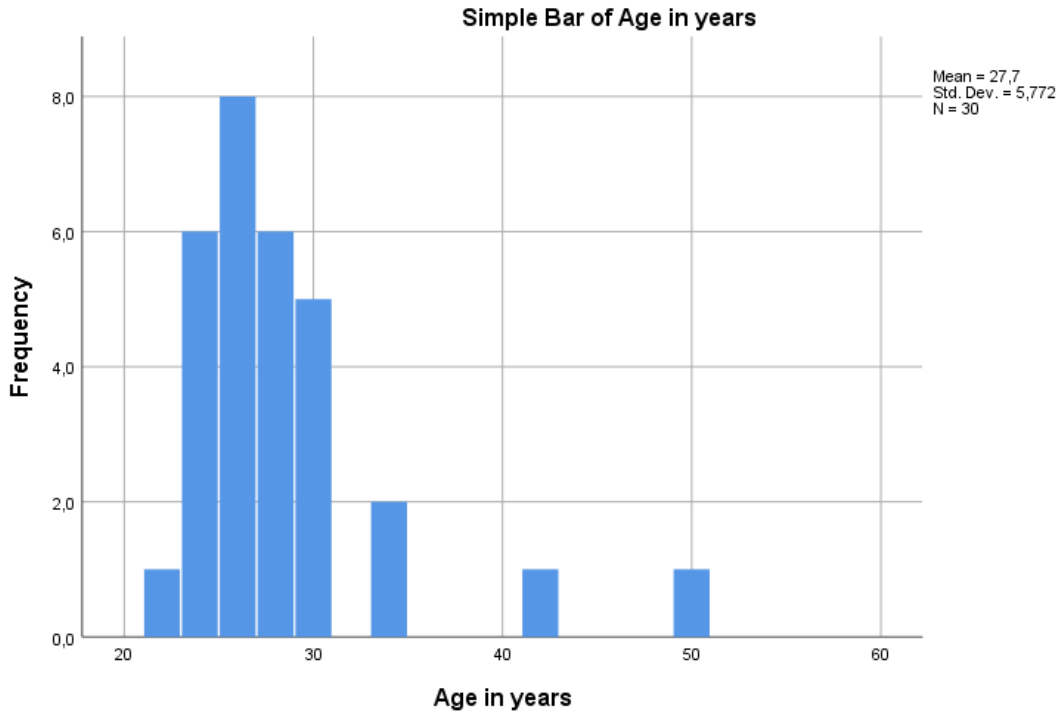


Figure 17 Mean age

➔ The mean age of the participant's was 27 years and 8 months

Sex

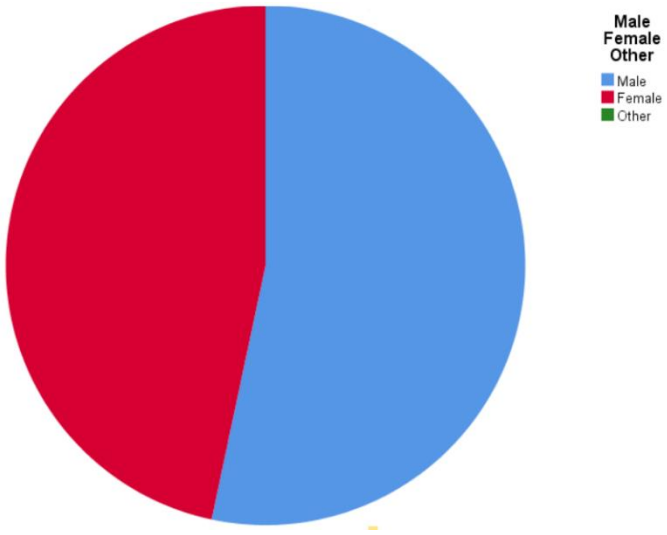


Figure 18 Male/Female

		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	Male	16	48,5	53,3	53,3
	Female	14	42,4	46,7	100,0
	Total	30	90,9	100,0	
Missing	System	3	9,1		

Total		33	100,0		

Table 51 Male/Female

➔ 16 out of 30 participants were male

Do you mostly work inside or outside?

		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	Only inside	18	54,5	60,0	60,0
	Mostly inside	9	27,3	30,0	90,0
	As much inside as outside	3	9,1	10,0	100,0
	Total	30	90,9	100,0	
Missing	System	3	9,1		
Total		33	100,0		

Table 52 Place of work

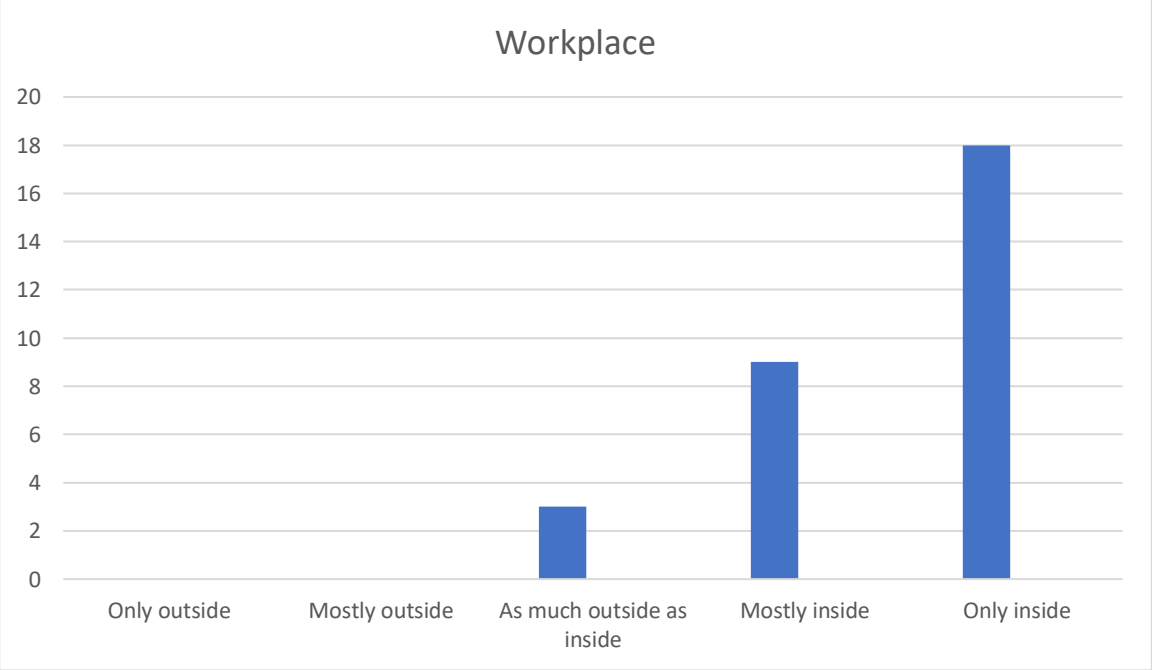


Figure 19 Place of work

➔ Most participants worked only inside

Have you already gone to a dermatologist for prevention or therapy?

		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	Yes	16	48,5	53,3	53,3
	No	14	42,4	46,7	100,0

	Total	30	90,9	100,0	
Missing	System	3	9,1		
Total		33	100,0		

Table 53 Dermatology visits

➔ Most participants had already gone to a dermatologist

Have you already gone to a dermatologist for mole screening?

		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	Yes	8	24,2	26,7	26,7
	No	22	66,7	73,3	100,0
	Total	30	90,9	100,0	
Missing	System	3	9,1		
Total		33	100,0		

Table 54 Mole screening

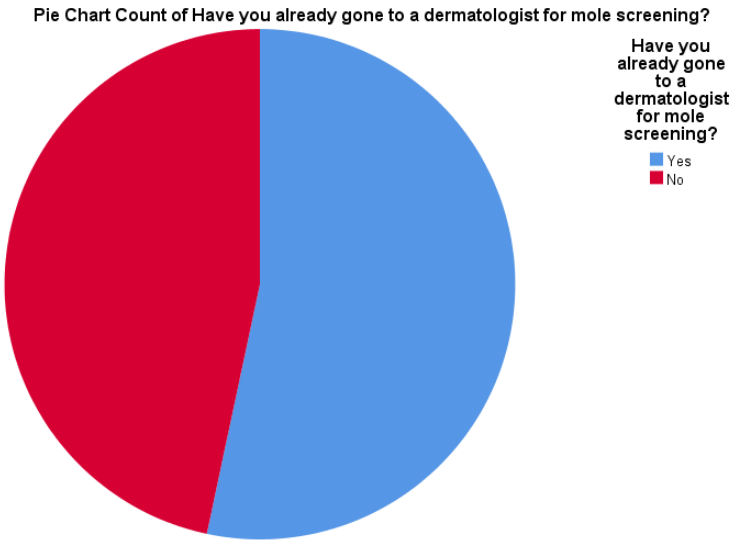


Figure 20 Mole screening

➔ Most participants had never had a mole screening performed to them

Conclusion to the epidemiological questions: the majority of the participants seems to be on the younger side and have a dermatological history. Considering that the starting age for a mole screening for most German state insurances is 35 years old, it is not surprising that many of the participants (average age close to 28 years) have never had a mole screening performed on them. There seems to be no significant difference in the gender of the participants as there was close to a 50/50 male/female split.

How convinced are you of the sun-protecting properties of the product?

		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	Very convinced	4	12,1	13,8	13,8
	Mostly convinced	18	54,5	62,1	75,9
	Neither nor	4	12,1	13,8	89,7
	Mostly not convinced	3	9,1	10,3	100,0
	Total	29	87,9	100,0	
Missing	System	4	12,1		
Total		33	100,0		

Table 55 Degree of conviction

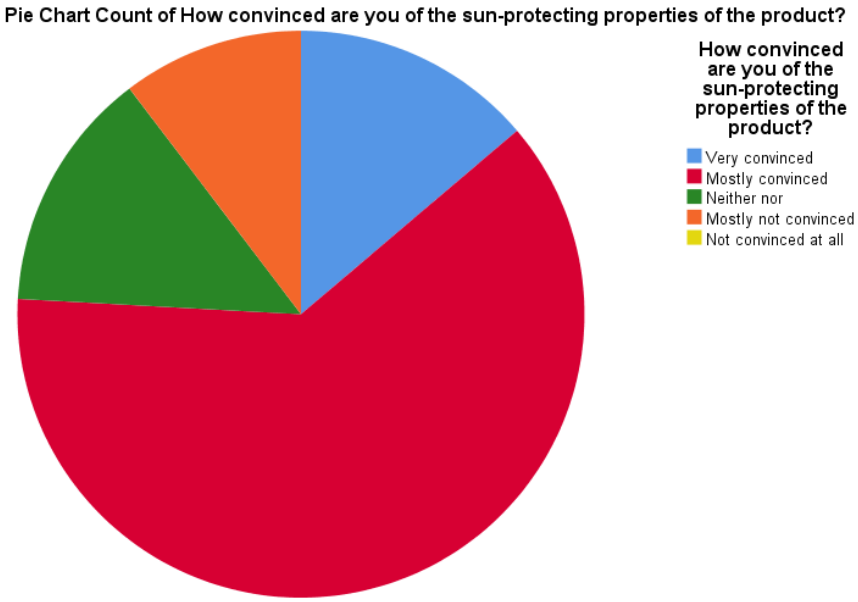


Figure 21 Degree of conviction

➔ Most participants were “mostly convinced” of the sun-protecting properties of the product

Would you recommend the product to a friend?

		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	Very likely	7	21,2	23,3	23,3
	Likely	13	39,4	43,3	66,7
	Unlikely	9	27,3	30,0	96,7
	Very unlikely	1	3,0	3,3	100,0
	Total	30	90,9	100,0	
Missing	System	3	9,1		
Total		33	100,0		

Table 56 Recommendation to a friend

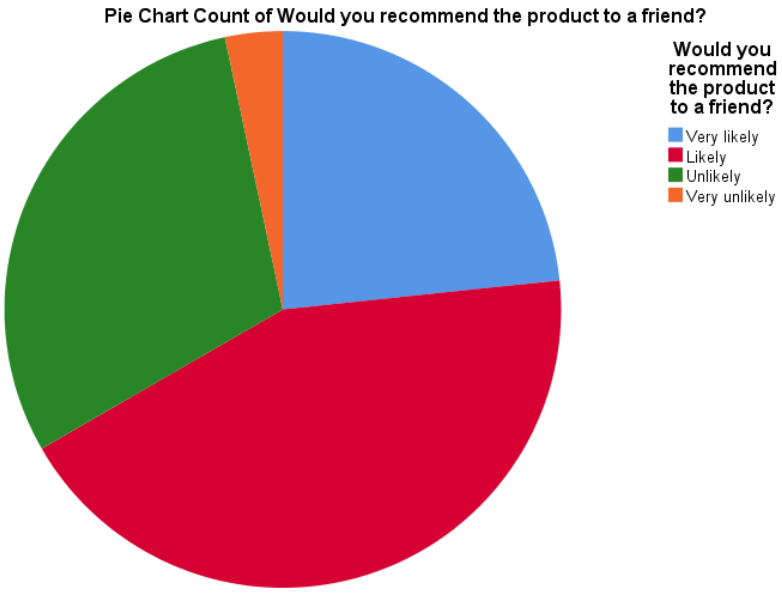


Figure 22 Recommendation to a friend

➔ Most participants would probably recommend the product to a friend

Would you buy the sunscreen?

		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	Yes	17	51,5	56,7	56,7
	No	13	39,4	43,3	100,0
	Total	30	90,9	100,0	
Missing	System	3	9,1		
Total		33	100,0		

Table 57 Buying decision

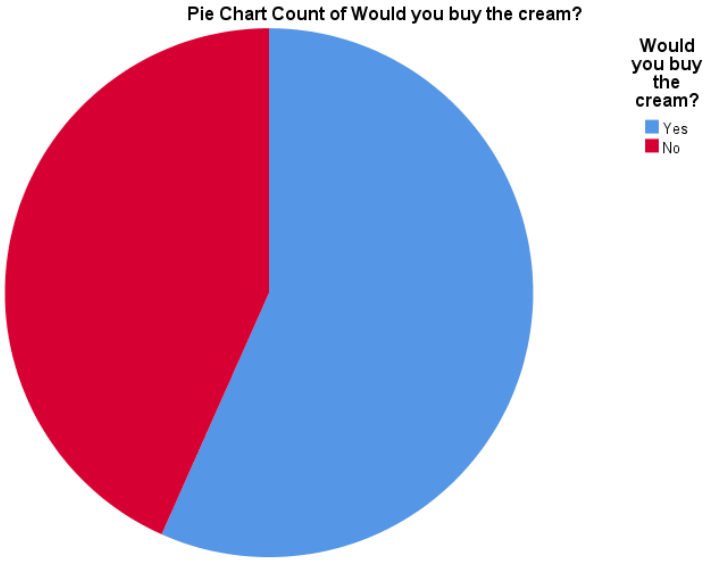


Figure 23 Buying decision

➔ Most participants would buy the product

How much would you spend for the product?

N	Valid	30
	Missing	3
Mean		10,5333
Median		10,0000
Minimum		,00
Maximum		25,00
Percentiles	25	6,5000
	50	10,0000
	75	15,0000

Table 58 Money participants would spend

Figure 24 Money participants would spend

➔ The mean that the participants would pay is 10,53 euros

What is your overall rating of the product?

		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	Very good	5	15,2	17,2	17,2
	Good	15	45,5	51,7	69,0
	Neither nor	7	21,2	24,1	93,1
	Bad	2	6,1	6,9	100,0
	Total	29	87,9	100,0	
Missing	System	4	12,1		

Total	33	100,0		

Table 59 Overall rating

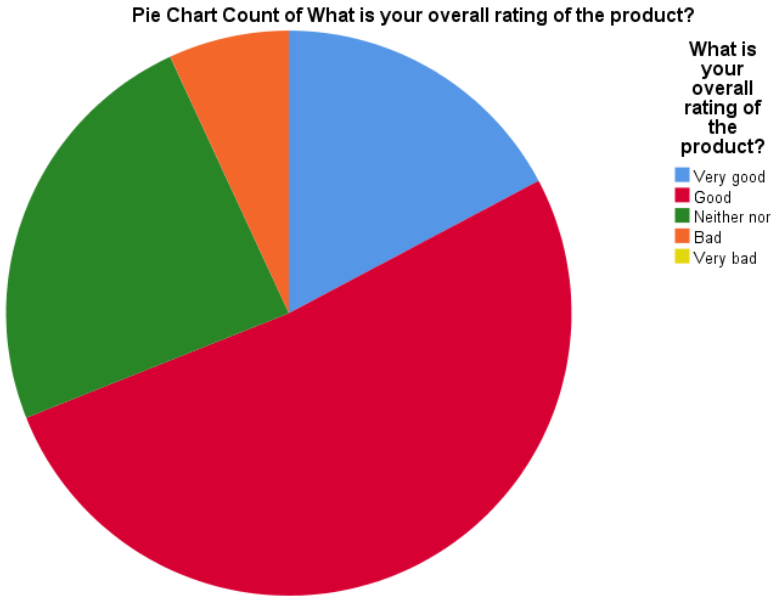


Figure 25 Overall rating

➔ Most participants rated the product as “good”

Conclusion to the sunscreen-specific questions: there seems to be an overall positive response of out participants to the use of the sunscreen in their daily skincare routine, which is relevant especially when compared to the objective measurements in the TEWL and SCH tests. Despite the sunscreen not being able to improve hydration and water-loss levels of the skin, the participants had an overall positive opinion of the product and would both buy it and recommend it to a friend.

Brown-coloring tests

All the shirts were examined on both sides for signs of brown coloring and product particles. 8 out of the 10 T-shirts showed no signs of brown coloring or product particles.

2 out of the 10 sunscreens had already been used for the TEWL and STH tests. Because of the higher density of the EGCG particles compared to the rest of the cream, it is possible that the EGCG sank to the bottom of the container and did not therefore get applied proportionally to the rest of the cream. This would lead to the "real" EGCG concentration being much higher than the reported 2%.

Figure 26: White T-shirt before (left) and after (right) application of sunscreen.

Two T-shirts showed signs of diffuse brown coloring. The brown marks presented themselves mostly in the crewneck, in the lower abdominal area and on the upper back and shoulders. The location of the brown coloring is not surprising as it matches the most accessible areas for sunscreen application. The degrees of brown coloring vary from 2 to 6 on the color palette. The areas with a 6th degree coloring match for both T-shirts (crewneck and low abdomen).

Figure 27 : First T-shirt which showed signs of brown-coloring

Figure 28: Second T-shirt which showed signs of brown-coloring

Sunscreen application dosage

In order to analyze the results IBM SPSS Statistics 26 was used. The amount of cream applied by the volunteers was compared to the amount they should have applied according to the European standards.

	N	Mean	Std. Deviation	Std. Error Mean
difference weight	10	5,7810	2,12670	,67252

Table 60 Weight difference

	N	Mean	Std. Deviation	Std. Error Mean
standard	10	19,9480	3,04550	,96307

Table 61 European standard

The mean weight of the applied cream was 5,78 g, the standard would have been close to 20 g. This show how the amount of product actually used under normal life condition is lower than the amount, which is used to test the sunscreen. The actual dosage per centimeter was 0,58 mg/cm².

Discussion

The goal of this project was that of developing a sunscreen which:

- Contains Epigallecatechin-3-gallate
- Takes advantage of the anti-inflammatory properties of the molecule
- Does not contain microplastics
- Does not contain nanoparticles
- Is 100% biodegradable
- Has a SPF of 50

These goals were set by looking at the environmental footprint of sunscreen products. It has been estimated that circa 14 million kilograms of sunscreen end-up in the ocean and sea water each year.

Since the beginning of the project there have been geopolitical changes which led other sunscreen producers to also invest in a 100% biodegradable microplastic-free sunscreen. As of January 1st 2021 sunscreen products, which might damage the marine fauna and flora cannot be purchased or brought onto the state of Hawaii. (CNN, 2018) Similar bans had already been applied in the United States Virgin Islands and in the island country of Palau. (Altmeyer & Barth, 2020)

More and more consumers seem to concern themselves with the ingredients of the products that they apply on their skin. Not only for the negative effects that harmful substances can have on their organism but also for their constantly increasing environmental footprint.

Most sunscreen products on the market and many other cosmetic products contain titanium dioxide (TiO2) or zinc oxide (ZnO). These two ingredients are mineral (or physical) UV-filters and they directly block and reflect UV-rays.

Titanium dioxide and zink oxide when exposed to UV radiation, form radicals, attack the DNA and can be harmful to the environment. In contact with water, they build highly reactive hydrogen peroxide. Hydrogen peroxide affects the growth of microorganisms such as phytoplankton. (Miller et al., 2012)

Benzones on the other hand are common chemical UV-filter. They are absorbed by the skin and do not reflect ultraviolet radiation, but they disperse it as low-energy heat.

The effects of these substances seem to not be confined to the marine world. The center of disease control reports that 97% of the tested participants showed traces of Oxybenzons in urine. Octylmethoxycinnamat and Oxybenzon have been shown in animal experiments to have hormonal effects. Some UV-filters have been also found in breast milk, which has raised concerns over their potential negative effects on the health of breastmilk-fed children. (Altmeyer & Barth, 2020; Schlumpf et al., 2008)

In the in-vitro testing a formulation was developed which met the above-mentioned criteria, did not exceed the 25% limit for titanium dioxide and zinc oxide did not contain any microplastics or nanoparticles and used only natural additives, while still being stable at both room temperatures and 40 degrees for weeks. Stability is of key importance, since sunscreen products are subjected to extreme temperature changes, and they must be able to keep their protecting properties under different atmospheric conditions.

A key part of the project was the literary research focusing on stability, interactions and the health aspects of the EGCG molecule. This was not only necessary to understand the properties of the polyphenol but also in order to be able to evaluate if the molecule can be used in the development of a sunscreen product.
The scientific data concerning molecule stability led us to believe that EGCG is more stable at lower temperature and pH values, while higher concentrations seem to slow down the degradation process.

The 0,0006 mM concentration performed worse than the 1,9651 mM one. The latter stayed stable for 48h at the temperature of 4 degrees Celsius, the former however degraded fully even though it was subjected to identical conditions. (Fangueiro et al., 2014)
In the interactions subsection many common cosmetic ingredients were reported to provide the EGCG molecule with better stability. In particular titanium dioxide (coated with EGCG) and vitamin C showed to inhibit the degradation process. Some cosmetic ingredients, vitamin C and E, even enhanced EGCG antioxidant properties. (Intra & Kuo, 2007; Scalia et al., 2013)

It should however be mentioned how the literature research for the “interaction” subsection revealed itself to be complicated by the fact that the existing literature was not very abundant. Further scientific research in this area is therefore needed.
The molecule has been thoroughly examined in the context of the treatment of many common dermatological conditions. Interesting results have been reported in the treatment of psoriasis, neurodermitis and UV-induced cutaneous damage.
EGCG performed well in the prevention of both UV-A and UV-B induced skin damage, the molecule has moreover important anti-inflammatory properties which can lead to tumor prevention and apoptosis. (Lu et al., 2002)
When compared to the “interactions” endpoint, the literature for stability and health aspects was abundant and detailed. Unfortunately, many of the studies used different endpoints and methods and it was therefore not always easy to compare them. Even when the papers had comparable endpoints, they would often lack standardized concentrations, temperature pH-values etc.… A more standardized approach to the scientific research of EGCG would lead to more comparable and replicable results.
Despite the abundant scientific literature on EGCG, the review was the first to analyze the properties of the EGCG molecule in the context of the development of a dermatologic and cosmetic product. However, the gold standard for publications of this kind is a systematic review. Therefore, further scientific research and the application of stricter criteria for systematic reviews are required.

The in vivo testing started with the selection of one sunscreen.
One of the 4 sunscreens was selected for further testing by means of questionnaires and the questionnaires results show how many volunteers were reported to be interested in more eco-friendly sunscreen products. More than half (53,9%) would buy the selected sunscreen (sunscreen 2). It should also be noted both sunscreens with a brown color reached higher selling prices, despite the fact that fewer people were inclined to buy them. The reason for this apparent inconsistency may lie in the color itself. Being that both products looked brown, it may have unconsciously led more participants to associate them with make-up products, which are, generally, more expensive than sunscreens. A handful of the participants asked, while filling out the questionnaires, if sunscreens 3 and 4 were make-up products, which would further indicate how either consciously or unconsciously many participants might have associated sunscreen 1 and 2 to normal sun protecting products and sunscreen 3 and 4 to make up products.
Overall, a very positive response to an EGCG-based eco-friendly sunscreen was reported, some volunteers showed some concerns regarding the smell of the cream, which was considered by many as “not sunscreen-like”. The reason for the “different” smell relies on the goal of developing a 100% biodegradable product and therefore not using artificial fragrance additives. It should also be noted that the sunscreen that was
picked for further testing had an overall positive response to the “smell” question. 65,4% of the people asked said that the sunscreen had either a neutral or a pleasant smell.

In the ultraviolet-light MED-tests it was reported how the mean difference in the cutaneous reaction intensity between protected and protected fields went from 0,31 to 5,68 for UV-B (from 25 mJ/cm² to 150 mJ/cm²) and from 0,46 to 0,85 for UV-A (from 5 J/cm² to 25 J/cm²). In particular the visible reaction to UV-A went from 0, for both protected and unprotected sides, to 0,2 for the sunscreen and to 1,3 for the no-sunscreen side. The data clearly indicated that the sunscreen is able to provide protection against UV-A/B light both at low and high radiation intensity.

Based on the previous in-vitro SPF tests and on the existing literature reporting on EGCG UV-protecting properties (anti-inflammatory, antioxidative, anticarcinogenic and direct sun protection) it can be said, with a reasonable degree of confidence, that the product can protect the skin against both UV-A and UV-B. (Camouse et al., 2009; Katiyar, Afaq, et al., 2001; OyetakinWhite et al., 2012; Vayalil et al., 2003; Yang et al., 2009) It is also important to notice how the protection is consistent despite the raising UV-radiation.

Nevertheless, further UV-B and UV-A MED tests should be performed on a larger sample size and include sunscreen products with no EGCG in order to assess the actual anti-inflammatory properties of the molecule after UV radiation. Another limitation of the MED-tests was the lack of skin-diversity of the subjects. The products were tested on individuals with a Fitzpatrick skin type I and II. While the population in Germany and Europe becomes more and more diverse, more tests are needed on the skin of individuals with skin types darker than III. Despite the fact that darker-skinned individuals are obviously more naturally protected from UV-damage, they can still be subjected to UV-induced skin damage.

A difference in RSOM values between fields 1 and 4, and between 5 and 8 was expected, since 1 and 5 were both protected and radiated with the lowest intensity and 4 and 8 were unprotected and radiated with the highest intensity. The RSOM data showed no difference in vascular response and since there is no other study using RSOM for the evaluation of UV-induced skin damage, other standardized parameters of testing need to be set before conducting similar experiments. For example, by using higher radiation intensities and multiple RSOM tests in the 24 hours post UV-exposure. Moreover, despite the many advantages which RSOM-measurements bring, the collected data is not always replicable and therefore difficult to compare.

In our transepidermal water-loss and stratum corneum hydration tests, the EGCG sunscreen not only did not improve TEWL and SCH of the participants' skin, but it seemed, at the end of the 28 days, to worsen their SCH values in both examined areas and to worsen their TEWL value on the arm of the participants. The decrease in the TEWL and SCH values was apparent and significant after the first two weeks but did not significantly worsen during the duration of the last two weeks of application. The EGCG sunscreen did not therefore show to improve skin hydration and water loss like many skincare products do.

There are a number of possible explanations for these findings:

1. The participants might have started applying the sunscreen instead of their previously used skincare product on the two body areas, which might have been a relevant cofounder
2. The EGCG particles used in the sunscreen might have been too dense and therefore sank to the bottom of the sunscreen bottle, the participants might have therefore unconsciously not applied the right amount of EGCG particles for them to have an anti-inflammatory and skin-caring effect
3. Due to the very liquid nature (some volunteers complained about the sunscreen not being dense enough) of the product there might have been some compliance problems from the participants, which might have led to inaccurate results
4. Some of the participants complained about the strong odor of the cream which might have led them to use less than the suggested doses (especially on the face) and therefore distorted the results

A more standardized test of transepidermal water loss and skin hydration is therefore needed. The pool of participants also needs to be more uniform and less subjected to external cofounders.

After TEWL and SCH the volunteers' opinion of the product was evaluated by means of questionnaires. 75,9% of the people asked were either "very" or just "convinced" of the sun protecting properties of the product. 2/3 would recommend it to a friend and 56,7% would buy it (10,5 euros was the average money they would spend on it). 69% found the product either "good" or "very good", volunteers were both satisfied with the product and convinced of its UV-protection to the point that they would buy it. Despite these promising results, the participants were obviously aware that they were using the EGCG-cream and this might have skewed the results. Further tests, involving a blinding process are needed.

As already mentioned in the review of the stability of EGCG, it has been shown in studies that the molecule tends to oxidate and its color changes to brown. This has been very apparent in the project in the selection of 1 of the 4 sunscreens by the 104 volunteers, since 2 out of the 4 sunscreens had the typical EGCG brown color. Despite the fact that the majority of the volunteers opted for one of the two sunscreens with a "normal" white color, brown-coloring tests on textiles were performed.

Out of the 10 T-shirts put on by the volunteers (who had previously applied the sunscreen) 8 had no brown marks at all. The problem that led to the brown-coloring of the resting two T-shirts was quickly identified. The EGCG-sunscreen containers, which had been provided to volunteers, had already been used for the TEWL and SCH tests and a good amount of the cream had already been consumed.

This represents a major problem and might affect the marketability of products containing EGCG and their acceptance amongst consumers.

The molecules of EGCG showed to be denser than the rest of the product and therefore sank to the bottom of the containers (figure 28). The EGCG concentrations in the two containers were much higher than it was supposed to be and was speculated to be the problem that led to the brown coloring. This problem was communicated to Systemkosmetik.

Figure 29: EGCG particles sediment that sank to the bottom of the container.

Previous studies have reported that many sunscreen users end up applying less than the advised 2 mg/cm² of sunscreen. It has been shown that the "real-life" amount used generally ranges between 0,5 and 1 mg/cm² and that many consumers, even after proper sunscreen application education, still end up using less than the advised amount. (Azurdia et al., 2000; Petersen & Wulf, 2014; Reich et al., 2009) The real-life sunscreen doses applied by the volunteers were tested. The findings were consistent with the preexisting literature, the volunteers used approximately 0,58 mg of sunscreen for cm². With these results in mind, it is easy to understand how even people, who use sunscreen regularly, might still be subjected to UV-induced skin damage and not know it.

The development of the first biodegradable sunscreen containing EGCG represents one of the first steps in a more environmentally aware approach to skincare and UV-protection. As previously mentioned, as of the first of January 2021 environmentally damaging sunscreens are banned in Hawaii. In a world which is incrementally becoming more and more aware of the man-made changes to the environment, it is reasonable to assume that more countries are going to follow. For these reasons preventing sunscreens-

induced pollution needs to become a goal of both governments and skincare production companies all over the world.

Summary

As the first step of the project, the scientific literature concerning Epigallocatechin gallate was examined with regard of three main endpoints: stability, interaction with common cosmetic ingredients and health aspects. Concentration, pH-value, temperature and mediums were reported to directly affect the polyphenol stability. Higher concentrations led to better overall stability. The same can be said for low temperatures and pH-values. At temperatures higher than 25-30 degrees Celsius and at pH-values over 4 the molecule shows to be very unstable and degrade quickly. In particular it is interesting to notice the degradation seems to occur by the two mechanisms of oxidation and epimerization. At pH-values between 4 and 8 both degradation mechanisms seem to occur parallelly.

After the literature research the goal was that of developing an EGCG-based sunscreen product with a SPF of 50 and containing no microplastic or nanoparticulate titanium dioxide or zinc oxide. Working with the cooperation partner of SystemKosmetik the goal was achieved and a sunscreen product with an SPF of 50 at a concentration of 18% titanium dioxide and 18% zinc oxide was developed. Four different versions of the creams with different consistencies and galenic were then produced to be tested for consumer satisfaction. 104 willing participants were recruited and presented with the four different versions of the products. After collecting the volunteers' satisfaction data, one of the 4 products was selected to be produced for further in-vivo testing.

10 willing (5 females and 5 males) participants were recruited, and 4 different MED-tests were carried out:

1. UV-B MED on an unprotected skin area
2. UV-B MED on a skin area protected with sunscreen
3. UV-A MED on an unprotected skin area
4. UV-A MED on a skin area protected with sunscreen

The results showed that the product was able to significantly protect the skin after both UV-B and UV-A radiation. Raster scan optoacoustic measurements were then performed on each of the radiation fields to be able to compare the different vascular reactions. Unfortunately, the imaging did not seem to show the cutaneous reaction previously measured with a chromameter.

After having proved the sun protecting properties of the sunscreen both in-vitro and in-vivo, the anti-inflammatory and antioxidant properties of Epigallocatechin gallate ware tested on human skin. 33 volunteers were recruited and instructed to apply the product on two specific skin areas at least once a day and transepidermal water-loss and stratum corneum hydration tests were performed over a 28 days period. The collected data over the for weeks did not show significant improvement in either the hydration levels or the amount of water that gets dispersed thorough the skin.

Consumer satisfaction with the product was evaluated by means of questionnaire. The 30 volunteers of the TEWL and SCH tests were asked to evaluate the product and their satisfaction on multiple choice questionnaires. The data shows that most of the volunteers were very satisfied with the EGCG sunscreen and would recommend the product. Some indicated they would prefer using it over their current sunscreen.

Ultimately, the issue of EGCG brown coloring and of sunscreen application dosage were tested in vivo. The molecule of EGCG is known for its tendency to quickly oxidate and change color, seeing how this might be an issue for a sunscreen product, we tested the product to examine if it would leave brown spots on white T-

shirts. (Krupkova et al., 2016; Sang et al., 2005) All but two T-shirts showed no signs of brown coloring. This apparent inconsistency can be attributed to a solubility issue in some of the sunscreen containers (which had previously already been used for TEWL and SCH testing), where the EGCG molecules were much denser than the medium and sank to the bottom leading to inconsistent concentrations. (Figure 10)

Most sunscreen consumer may not be using as much sunscreen as it is advised. The tests showed that all but one volunteer used less than half then the advised doses and were therefore not appropriately protected.

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