Master's Thesis

# Development and Evaluation of an Enzyme-Based Sunscreen

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# ABSTRACT

Ultraviolet radiation (UVR), specifically of UVA and UVB, has been associated with the development of both photoaging and photo-carcinogenesis, potentially leading to cancer. The use of sun protection in the form of UV filters is known to minimize these damages. By introducing an enzyme-based sunscreen for daily facial use these damages may be reduced while also providing exfoliation and hydration to the skin. Enzymes as exfoliating agents are gentle compared to mechanical or chemical exfoliating agents and in combination with inorganic UV filters, which in turn are considered more gentle than organic UV filters, this will minimize the risk of skin irritation and allergic reactions. The primary objective of this project is to develop a product with favorable cosmetic properties while maintaining enzyme activity and stability, as well as protecting against a broad spectrum of UVR.

Multiple formulations were prepared, each containing protease enzymes and inorganic UV filters and were each evaluated based on the parameters mentioned above. Enzyme activity was assessed by measuring the amount product produced by the of hydrolysis of substrate during a defined time period using a protease assay. The absorbance and transmittance of the formulations were measured to assess their efficiency in shielding against the UV wavelengths. Formulations demonstrating low enzyme stability or unpleasant sensational characteristics were gradually excluded from further development. Consequentially, three samples were further evaluated in the sense of customer satisfaction by volunteer participants testing the samples and answering theoretical and practical questions to gain a better understanding of which formulation to proceed with and potentially introduce to the market. Taking in all aspects evaluated the formulation containing UV filter Z1 has been proved to be the most preferred.

# Popular Science Summary

Spending time in the sun can be a wonderful experience, however, it is important to remember how damaging solar radiation can be to our skin. Too much exposure to the ultraviolet radiation (UVR) from the sun can cause damage to your skin in the form of premature aging and wrinkles along with an increased risk of skin cancer. Protecting yourself from this damaging UVR in the form of protective clothing or sunscreen is therefore crucial. Sunscreen contains special ingredients called UV filters shielding solar radiation from penetrating our skin. There are two main types of UV filters: organic (chemical) filters, which work by absorbing the UVR and converting it into heat, and inorganic (physical) filters which can both absorb but also reflect and scatter the UVR.

This project strives to develop a daily facial sunscreen while incorporating enzymes into the formulation. Enzymes are biomolecules which help speed up chemical reactions in living organisms. Specifically, the enzymes used in these formulations are called proteases, which break down proteins into smaller parts. Proteases used in cosmetics are often used as exfoliating agents to break down and remove proteins from the skins surface, imitating the natural shedding of dead cells to make the skin smooth and soft. By combining the protective characteristics of sunscreen with the potential benefits of the enzymes this project seeks to create a product which not only minimizes the harmful effects from solar radiation but also incorporating a gentle enzyme based exfoliating action while providing skin hydration.

# TABLE OF CONTENT

# ABBREVIATIONS

| | |
|---|---|
| CSAR | Cosmetic Supervision and Administration Regulation |
| ECM | Extracellular Matrix |
| FDA | Food and Drug Administration |
| $Fe_2O_3$ | Iron Oxide |
| GDB | Glycerol Dilution Buffer |
| GMP | Good Manufacturing Practice |
| GRASE | Generally Recognized as Safe and Effective |
| IRA | Infrared Radiation |
| NDA | New Drug Application |
| NMPA | National Medical Product Administration |
| OTC | Over the Counter |
| p-NA | Para-Nitroaniline |
| ROS | Reactive Oxygen Species |
| SPF | Sun Protection Factor |
| STSC | Safety and Technical Standards for Cosmetics |
| $TiO_2$ | Titanium Dioxide |
| UVR | Ultraviolet Radiation |
| VIS | Visible Light |
| ZnO | Zinc Oxide |

# 1 Introduction

The diagnosed cases of skin cancer have increased significantly over the past few decades, and this is predominantly due to excessive exposure to solar radiation [1]. The fact that ultraviolet radiation (UVR) causes damage to our skin both in the form of photoaging and photo-carcinogenesis is widely acknowledged, however, the extent of damage and how to, most efficiently, protect yourself from UV radiation while simultaneously minimizing the environmental damages caused by various UV filters remains a challenge which will be discussed in this report.

Exposure to the sun in a moderate amount has been proven to be beneficial in the sense of enhanced vitamin D production, improved cardiovascular health and antimicrobial activity [2]. However, excessive sun exposure can cause both photoaging and sunburn and ultimately cancer. Hence, the use of sun protection, such as e.g. textile protection or sunscreen, is of great importance [1].

Sunscreens are formulated as creams, lotions or sprays containing a range of UV filters, which function via diverse mechanisms such as absorption, reflection and scattering of the UVR emitted from the sun. There is a wide assortment of UV filters on the market. They differ from each other in their chemical composition and the mode of action by which they protect the skin from UVR. Finding the right one might be a challenging task. In addition, some UV filters have harmful environmental effects, such as pollution and bleaching of corals, due to the large quantities are released into the seas [2]. Consequentially, avoiding such agents is imperative.

This project aims to develop an enzyme-based moisturizer that incorporates UV filters, commonly known as sunscreen, where the enzyme is both active and stable within the product while simultaneously presenting efficient protection from the solar radiation considering both the UVA and UVB spectra.

# 2 Background

Regardless of geographical location, protecting yourself from solar radiation is crucial. UVR stretches between the wavelength of 100-400 nm and comprises UVA (320-400 nm), UVB (280-320 nm) and UVC (100-280 nm). UVC is almost entirely absorbed by the ozone layer, leaving UVA and UVB as a greater threat for skin damage [3]. These diverse types of radiation

have been linked to photoaging and photo-carcinogenic effects, therefore, it is crucial to stay out of the sun during peak hours and to wear appropriate sun protection [1].

## 2.1 PHOTOAGING

Photoaging is a result of excessive exposure to UVR from the sun, particularly from UVA radiation. When the skin is directly exposed to UVR this can result in a variety of defects [4]. The skin is the human's largest organ, composed of numerous layers, where the epidermis and dermis are the two primary layers. The dermis, located beneath the epidermis, is the layer that is damaged the most from UVA radiation, as UVA penetrates through the epidermis into the dermis [3]. The dermis is a connective tissue and consists mainly of fibroblasts, which are responsible for producing collagen, elastin, fibrin and other proteins in the extracellular matrix (ECM). These proteins are responsible for the skin's characteristics making it elastic and resistant, and damage to these proteins can lead to accelerated aging of the skin and the formation of wrinkles [5].

When the skin is exposed to UVR, especially UVA radiation, reactive oxygen species (ROS) can be generated which can have clinical, histological and biochemical effects on the skin [6]. ROS can result in oxidative stress, leading to apoptosis or necrosis. An excessive amount of generated ROS can degrade and damage proteins in the skin, including collagen and elastin and can also act as a secondary messenger causing mutations in the DNA, ultimately affecting the transcription and expression of certain genes [4]. These genes may play a role in autophagy, a process in which abnormal or damaged proteins are degraded and destroyed. By mutating these genes, these mechanisms can be inhibited leading to photoaging as a result of DNA damage caused by UVR [3].

## 2.2 PHOTO-CARCINOGENESIS

Exposure to UVR is not only associated with skin aging but also with more severe damages such as cell mutation, immunosuppression and the development of various types of skin cancer including basal and squamous cell cancer and melanoma. Among these, the risk of skin cancer formation is the most concerning. Unlike photoaging, which is mainly connected to UVA radiation, these photo-carcinogenic effects are mainly attributed to exposure to UVB [7]. However, UVA is much more abundant in sunlight, and accounts for approximately of 90% of UVR, and can therefore contribute to some photo-carcinogenesis despite its lower carcinogenic potential [8, 9].

Unlike UVA, which primarily targets the dermal layer of the skin, UVB is associated with the epidermal layer composed mainly of keratinocytes. UVB radiation can suppress the immune system by inducing the release of immunosuppressive mediators, such as cytokines, derived from the keratinocytes or by inhibiting antigen presentation. Experimental data has demonstrated that immune suppression can contribute to skin cancer development in mice, and likely in humans as well. Thus, individuals exposed to excessive sunlight are chronically immunosuppressed, which in turn can result in increased rates of skin cancer [8].

In contrast to UVA which generates ROS which in turn damages the DNA, UVB radiation can generate ROS as well but can also be absorbed directly by the DNA due to its shorter wavelengths which can lead to cancer via two different mechanisms. It can either cause damage or create mutations directly to the DNA by exiting the nucleobases, eventually leading to carcinogenesis, or it can mutate tumor suppressor genes making them inactive or dysfunctional, inhibiting them to suppress tumor progression [9, 10]. Additionally, UVB can also cause severe inflammation and erythema, more commonly known as sunburn, which shows a close correlation with carcinogenesis caused by DNA damage [11]. It is commonly thought that cancer is a result of severe sunburns. This is partially true, but it is important to acknowledge that sunlight can cause cancer even without the previous erythema and sunburns [10, 11].

## 2.3 Protection Against UV Radiation

Fortunately, there are several ways to protect the skin from the damage caused by UVR. This can be done by using different types of photoprotective agents such as UV filters. There are several classifications of these agents, for instance, naturally occurring photoprotective agents e.g. ozone, clouds and pollutants in the air, and physical photoprotective agents e.g. clothing, hats and sunglasses, however, this report will mainly be focusing on UV filters as an ingredient in sunscreens [11]. Sunscreens come in various forms including creams, lotions or sprays, but they all share a common characteristic of containing UV filters. These filters can be classified as either organic (physical) or inorganic (chemical) [1]. Effective UV filters should possess certain physical, chemical and biological properties, including the ability to protect against a broad range of UVR wavelengths, photostability, non-toxicity and compatibility with cosmetic formulations to become a successful sunscreen both within the sense of sales and skin protection [12].

### 2.3.1 Inorganic Filters

Inorganic filters function primarily by absorbing UVR, converting it to low energy radiation and heat, which is not considered harmful to the skin, and to some extent also reflecting and scattering UVR [1, 13]. Reflection and scattering are the main mechanisms that differentiate inorganic UV filters from organic. The inorganic filters thus provide increased protection against UVR as well as they have a lower risk of skin irritation along with lower negative environmental effects than organic UV filters [14, 15]. The most commonly used inorganic filters contain titanium dioxide ($TiO_2$) or zinc oxide (ZnO). The amount of reflected UVR depends partially on the size of the particles and the thickness of the coating, larger particles and thicker coating increases the reflection and scattering, however, reduces the cosmetic acceptability due to the formation of an unaesthetic white layer on the skin [11]. Iron oxide ($Fe_2O_3$) can e.g. sometimes be used to add a hint of brown color to the sunscreen to disguise the white coat [14]. By reducing the size of the particles through techniques such as grinding or milling, the reflective index decreases, while absorbance increases for shorter wavelengths. Consequentially, the protection range shifts, with less scattering and UVA protection, but more absorbance and higher protection from UVB, as well as an improved cosmetic appearance [1, 11]. However, too small particles such as nanoparticles, defined as between 1-100 nm, should be avoided as they can have negative impacts within manufacturing on the internal organs, as the particles can transfer into the airways of the factory personnel and the concentration of nanoparticles in the alveoli increases, possibly leading to absorption in the bloodstream. The risk of inhaling these particles can also impact the users of the sunscreens, on a smaller scale, and as a result of the inhalation risk, products in powder form or spray sunscreens containing ZnO and $TiO_2$ are not recommended [16].

The potential risks associated with the use of inorganic filters are considered to be low for both humans and the environment, they are generally considered lower than for the organic UV filters [16]. Some studies suggest that ZnO has a greater negative impact on the environment compared to $TiO_2$ in general, as ZnO has a higher solubility in water, and will therefore more easily release its $Zn^{2+}$ ions [17]. ZnO does however provide a broader spectrum of protection and protects more efficiently from both UVA and UVB radiation while $TiO_2$ primarily protects against UVB, this however also depends on the size of the particles, as mentioned above [15]. The environmental impacts of these filters include coral bleaching, toxicity in aquatic wildlife, including bioaccumulation, aggregation and the release of metal ions. However, further studies are necessary to draw any conclusions [16-18]. To avoid some

of the negative consequences of nanoparticles named above, platelet-shaped micron particles can be designed. As these particles are composed of nanoparticles, they have similar chemistry and the desired characteristics, while having decreased negative effects on both environment and human health. The larger size of these particles reduces the penetration risk into the cells [19].

ZnO and TiO2 are known to be photoreactive and may react with the UVR forming free radicals, ROS, which could potentially damage the DNA. To prevent this, the particles are often coated with materials such as silica, alumina or dimethicone [11, 14]. Sunscreens need to maintain their photostability to provide proper photoprotection. The less photostable the UV filters are the faster they will be absorbed and degraded when exposed to UVR exposure. Inorganic UV filters, in general, are more photostable than organic filters, making them advantageous for use in sunscreens [11].

### 2.3.2 Organic Filters

Organic UV filters are characterized by their absorption mechanism, as opposed to inorganic UV filter which also reflect and scatter UVR. The absorption mechanism works in the same way as for the inorganic filters, as they both transform the UVR into heat and emit low energy radiation. However, the organic filters are less photostable compared to inorganic filters and can undergo chemical conformations when exposed to UVR which can lead to degradation of the molecules [14]. Organic UV filters are often used in combination due to the permitted level of a single active ingredient is not high enough to reach a desired sun protection factor (SPF) but also to be able to protect against a broader spectrum of the UVR. However, a combination of different organic filters can result in decreased photostability [12, 14].

Organic UV filters have been shown to cause several adverse effects, including skin irritation, allergic reactions, and phototoxicity [11]. Furthermore, they are very difficult to remove from wastewater, and they have additionally been shown to cause negative environmental impacts on marine organisms and contribute to coral bleaching since large amounts end up in the sea [1, 15, 20]. Certain organic UV filters, such as oxybenzone and octinoxate, have even been banned in various regions worldwide, including Hawaii, Key West: Florida, Mexico and the Western Pacific nation of Palau, due to their negative impact on the environment [21]. Given these reasons concerns, sunscreen formulation efforts within this project will mainly focus on inorganic UV filters.

## 2.4 Global Market

The current market for sunscreens is influenced by multiple factors. There are several regulations and requirements to consider which may vary across different regions worldwide. Moreover, customer satisfaction is an important aspect to consider in combination with the regulatory guidelines to attain a successful product in the global marketplace.

### 2.4.1 Regulations and Requirements

The regulatory framework of sunscreen varies across different regions of the world. Regarding regulations worldwide, the European Union (EU), the United States (USA) and China represent the major areas of interest due to their significant impact on regulatory practices in other countries. Therefore, these areas will be focused on in this report.

Within the EU sunscreens are considered as cosmetics and are regulated by the Cosmetics Regulation (EC) No 1223/2009, which is a regulatory framework for all cosmetic products put on the market in the EU. This regulation provides guidelines for which UV filters are permitted and their maximum allowed concentration. As of today, around 30 UV filters are approved for use in the EU. The regulation considers potentially hazardous substances and safety assessment of various chemicals along with the responsibilities and accountabilities for safety regarding human health. It also includes matters such as manufacturing, labeling and packaging of cosmetic products [22]. Environmental safety aspects of substances are however addressed in a different regulation, namely Regulation (EC) No 1907/2006 [23]. The regulatory documents provide guidelines to ensure that legal requirements are fulfilled, however, it is the company, or so-called “responsible person”, who carries the responsibilities and ensures that all legal requirements are implemented before a product is placed on the market. Concerning the inorganic filters, which are the primary focus of this report, both ZnO and $TiO_2$ are permitted at concentrations of up to 25%, with some exceptions in the case of their combination with other UV filters. [24].

The USA differs from the EU and most of the world regarding the regulation of sunscreens as it is considered as an over the counter (OTC) drug according to the Food and Drug Administration (FDA). As of 2020, there were 16 approved UV filters by the FDA, however, there is now a New Drug Application (NDA) process in place to classify UV filters as Generally Recognized as Safe and Effective (GRASE) or not [20]. Of the 16 already approved UV filters, only two are classed as GRASE, namely ZnO and $TiO_2$. Two are classed as “not GRASE” which will no longer be permitted in sunscreens if they cannot achieve this

status within the given timeline, and the rest were classed as “insufficient safety data to confirm GRASE” requiring further study, though still permitted for use at present. The FDA regulations include requirements for labeling, safety studies and testing procedures. Additionally, certain states in the USA may have specific regulations for particular UV filters regarding labeling or even prohibiting their use. Several other UV filters, already approved in the EU are currently awaiting further testing by the FDA, though this process is slower than the EU’s approval process and may require additional time [25].

In 2020, China published a new regulatory framework for cosmetics, known as the Cosmetic Supervision and Administration Regulation (CSAR), which classifies sunscreens as so-called “special cosmetics”. This implies that they are registration regulated as well as they need to gain approval in the form of technical evaluations from the National Medical Product Administration (NMPA). The Safety and Technical Standards for Cosmetics (STSC), first published 2015, contains lists of permitted and prohibited substances, along with their respective limits and requirements [26].

In addition to these named regulations, several additional requirements and regulations exist for cosmetic products that must be met prior to their placement on the market. Such requirements may for instance include labeling and packaging. Moreover, all products should be produced according to the guidelines regarding good manufacturing practice (GMP) of cosmetic products [24].

### 2.4.2 Customer Satisfaction

Introducing a new product into the market, such as sunscreen, presents various challenges, alongside with research, formulations and regulations there is also the aspect of customer satisfaction. Despite the product’s functionality, environmental safety, reasonable pricing, etc. if the customer is not pleased with the sensory aspects or appearance of the product, it will not sell. However, what customers express that they want in theory with what they actually buy does not always align. It is therefore important to study and understand the market and demand both theoretically and practically to ensure a successful product placement.

## 2.5 Chemicals/Ingredients

When formulating a cosmetic moisturizer, there are certain ingredients that are commonly used. Among these, except for water, glycerin is one of the most prevalent as it helps the outermost skin layer, the stratum corneum, to maintain hydration by attracting water from the dermis and

epidermis out to the stratum corneum. Glycerol also improves the barrier function of the skin, thereby preventing the evaporation of water attracted from deeper skin layers. [27, 28]. Apart from glycerin, or glycerol, in combination with several other ingredients and the UV filters used in these formulations, there are two ingredients that are especially important in the formulation of this sunscreen. These are the proteolytic enzymes and the thickening agent, which will be discussed further in detail below.

### 2.5.1 Proteolytic Enzymes in Skincare

Proteolytic enzymes, or proteases, are primary responsible for cleaving peptide bonds, which can result in either inhibition, activation, regulation or loss of function of proteins [29]. Proteases are involved in several activities in the skin including desquamation (i.e. shedding of dead cells from the skin's surface), permeability of the epidermis, dermis and stratum corneum [30]. The use of proteases in skin care formulations has become increasingly popular due to their beneficial effects on the skin. While historically proteases have been used to debride necrotic tissue from wounds, their use in dermatological applications has evolved and they are now commonly found in formulations for skin cleaning, moisturizing and improving both the appearance and characteristics of the skin [29]. Proteolytic enzymes exfoliate the skin and mimic the desquamation by hydrolyzing peptide bonds in proteins such as keratin and desmosomes [30, 31]. The exfoliation triggers a cascade of reactions which have a healing effect and facilitates the repair of the skin, including the renewal of fibroblasts and keratinocytes and structure reorganization of the skin [30, 32]. In addition, these enzymes also cleanse the skin's surface of excess microbes, which can then be more easily removed when washing the skin [30].

Given the varying conditions on the skin, it is important to choose enzyme carefully based on the formulation's intended purpose. Enzymes are generally sensitive to pH and temperature which enables them to first be activated upon contact with the skin, triggered by the pH and temperature of the human body. Both the pH and the temperature of the skin can vary depending on environment, body part, lifestyle etc. The average pH value of the skin on the face ranges from 4.7-5.9. Factors such as washing your face with water, or with a cleanser or alkaline soap directly before application of the exfoliating enzymes can also influence the skin's pH [30]. Using enzymes as exfoliating agents is considered gentler than other exfoliation techniques such as chemical or mechanical exfoliants, partially due to the fact that enzymes are specific and only act where desired [30, 33]. Over-exfoliating the skin can lead to redness, irritation and sensitivity, this risk is greater when using chemical or mechanical exfoliants,

which are considered more harsh, rather than enzymes [33, 34]. Chemical exfoliants may include hydroxy acids which can be used in cosmetic formulations to achieve beneficial effects such as improvements on photoaged skin. However, studies have shown that when used in combination with extensive sun exposure, these products may increase sensitivity towards UVB radiation, leading to sunburn and increasing the need for sun protection. Consequentially, recommended limitations for the use of certain hydroxy acids have been introduced [35, 36].

Despite the advantages considering enzymes in skincare, several challenges must be addressed. Their requirement for specific conditions can be an obstacle in some cases, for instance if specific activators or inhibitors are absent, or if formulations are exposed to temperature outside of the optimal range. Enzyme stability can also be an obstacle to overcome as other ingredients in the formulations may bind to the enzyme or its active site, thereby decreasing or inactivating the enzyme activity. There is also a risk of irritants, allergic reactions or hypersensitivity to certain enzymes [30]. A study was conducted to verify the proteolytic activity level of 11 different enzymatic cosmetic products. Only two of these demonstrated sufficient amount of proteolytic activity to be able to be classed as enzymatic formulations and six of them showed no activity at all, indicating the difficulty associated with incorporating active enzymes within cosmetic formulations [34].

### 2.5.2 Thickening Agent

Carbomers are widely used in the cosmetic and pharmaceutical industries as thickening agents, they are hydrophilic cross-linked acrylic polymers with a high molecular weight creating gel-like products. Carbomers are typically powders that swell upon contact with water, as they undergo hydration. They contain carbocyclic acid groups, making them weak acids. To attain the gel-like structure they require neutralization with a base, consequently, this also makes them sensitive to changes in pH [37]. The gel may collapse if excessive base or salt is added, or insoluble complexes may form if divalent ions are added, since these can bind to the carboxyl groups [38]. Different carbomers can have varying thickening performances and attain different tolerance to electrolytes and changes in pH. Their differences depend partially on their variations in structure, composition and crosslink density [39, 40].

Cellulose serves as an alternative rheology modifier, it is a natural, sustainable and biodegradable material making it less harmful towards the environment compared to other synthetic thickening agents. Cellulose has a high water retainability and good stabilizing capacity and by absorbing water the cellulose can swell and form a gel like structure. Cellulose

can be used instead of a carbomer since it is less sensitive to pH variations and divalent cations due to its lack of carboxylic acids [40]. Xanthan gum is also a thickening agent option and it is a natural polysaccharide with similar properties to cellulose. It is a biopolymer and its rheological characteristics are relatively unaffected by changes in temperature and pH, making it suitable for formulations where such parameters may shift during storage [41].

# 3 Materials and Methods

This study contains the key steps involved in the development of an enzyme-based sunscreen. The primary objective is to ensure enzymes activity and stability within the product, while also providing an efficient protection against both UVA and UVB. Initially sunscreens containing various UV filters were formulated and the enzyme activity was measured. After determining which UV filters to proceed with, their absorbance and transmittance was measured within the wavelength range for UVR, along with repeated measurements of the enzyme activity to determine the enzyme stability. Evaluations regarding customer satisfaction were performed to identify the most promising product for potential success on a future market. Additionally, an assay was planned to investigate the generation of ROS of sunscreens when exposed to UVR, however, due to delays in the arrival of the required chemicals, there was insufficient time to perform this assay within the timeframe for this project.

## 3.1 Formulation of Moisturizer and Sunscreens

The formulation of the base moisturizer, which does not contain any UV filters, was prepared according to a recipe provided by ZymIQ Technology AB. This recipe included, among other ingredients, a carbomer serving as a thickening agent, and the proteolytic enzyme. Prior assessments of this moisturized had been conducted including evaluation regarding consistency, visual appearance as well as enzyme activity and stability within the formulation. To incorporate the UV filters, and achieve the desired SPF, the moisturizer was mixed with different UV filters received. The amount of each specific UV filter required to attain a particular SPF was determined using the manufacturer's SPF calculator provided on their website. This provided a range with an upper and lower inclusion level of the UV filters, indicating the necessary quantity. The lower inclusion level was used to estimate the minimum amount of UV filter needed to achieve the desired SPF, which in this case was SPF 15. The lower inclusion level varied across the different UV filters, ranging from 5-15%.

All sunscreen formulations were made in 500 ml beakers in batches of 100 g. The UV filters came in various forms, including emulsions, dispersions and powders. Table 1 provides a description of the eight UV filters that were tested in this project.

**Table 1:** List of UV filters tested and a short description.

| UV filter | Description | Average particle size (nm) |
|---|---|---|
| Filter Z1 | Uncoated powder dispersion containing ZnO | 604 |
| Filter Z2 | Uncoated powder dispersion containing ZnO | 604 |
| Filter T1 | Oil-based $TiO_2$ dispersion | 126 |
| Filter T2 | Triglyceride-based $TiO_2$ dispersion | 126 |
| Filter Z3 | Silane coated ZnO powder | 340 |
| Filter T3 | Alumina coated $TiO_2$ powder | 72 |
| Filter T4 | Alumina coated $TiO_2$ powder | 126 |
| Filter T5 | Water based $TiO_2$ dispersion | 201 |

Regarding the formulations involving UV filters supplied as dispersions (Filter Z1, Z2, T1, T2 and T5) the base moisturizer was prepared separately, followed by the addition of the respective UV filters to create the sunscreen formulation. However, for UV filters received in powder form, a different approach was used. These powders were initially mixed into a paste with glycerol and pentylene glycol in a fume hood to minimize the inhalation risk of the powders and to facilitate the mixing process. Subsequently, this paste was combined with the remaining ingredients for the moisturizer. After testing all UV filters, the sunscreens were evaluated, and modifications were implemented to improve the formulations. The alterations primarily involved exploring various thickening agents and applying heat during the formulation process. The new thickening agents tested included a different carbomer, cellulose and xanthan gum. Moreover, for formulations containing Filter T1 and T2 were mixed with the glycerol and pentylene glycol, similar to the powders, and heated to 70°C in a water bath on a heating plate. This heated mixture was then blended with the remaining ingredients, which were also heated to 70°C to attain a more homogenous mixture. This procedure was also tested for Filter T5. Table 2 provides an overview of all the formulations created with their respective combinations, one batch of each combination listed below was formulated.

**Table 2:** Different combinations for formulations of sunscreens.

| UV filter | Thickener | Heated |
|---|---|---|

| | | |
|---|---|---|
| Filter Z1 | Carbomer 1 | No |
| | Cellulose | No |
| Filter Z2 | Carbomer 1 | No |
| | Cellulose | No |
| Filter T1 | Carbomer 1 | No |
| | Carbomer 1 | Yes |
| | Cellulose | Yes |
| Filter T2 | Carbomer 1 | No |
| | Carbomer 2 | Yes |
| Filter Z3 | Carbomer 1 | No |
| | Cellulose | No |
| Filter T3 | Carbomer 1 | No |
| Filter T4 | Carbomer 1 | No |
| Filter T5 | Carbomer 1 | No |
| | Cellulose | No |
| | Xanthan | No |
| | Xanthan | Yes |
| | Cellulose + Xanthan | No |

All sunscreens created were stirred into a homogenous mixture in the beakers, using a small propeller agitator with a rotational speed of 300 rpm. Subsequently, these formulations were tested and evaluated with regard to their appearance and sensation. The evaluation included both individual assessments as well as application to the skin along with measurements of enzyme activity and a comparative analysis among the different sunscreen formulations.

## 3.2 Protease Assay

The enzyme activity of the sunscreens was measured at various time points: immediately after formulation, after one week and approximately 1-1.5 months of storage. The sunscreens were stored in Falcon tubes and subjected to two different storage conditions: one at room temperature (RT) in daylight and the other at 40°C in the dark, respectively, to determine the stability of the enzyme. A colorimetric assay was used to measure the enzyme activity. The color intensity of the solution is proportional to the cleavage of the substrate, para-nitroanilide, by the enzyme, hence, the amount of produced product, para-nitroaniline (p-NA). This makes it possible to determine the enzyme activity by assessing the rate of color formation. The measurement was conducted using a microplate reader, Multiskan Go (Thermo Scientific), set to 30°C and the absorbance was measured at a wavelength of 405 nm, which is the optimal

wavelength for product detection. A water bath (Julabo) was also set to 30°C ± 0.5°C and a 15 ml Falcon tube containing the substrate solution (containing para-nitroanilide and a buffer) was placed in the water bath to ensure consistent temperature conditions throughout the assay.

To prepare samples for the assay, the density of the sunscreens was determined to be 1.2 g/l. Eppendorf tubes containing 0.12 g (100 µl) sunscreen were diluted with 400 µl glycerol dilution buffer (GDB) to attain a dilution ratio of 1:5. Samples that did not contain a clear solution were centrifuged (Eppendorf, Centrifuge 5424) at 5000 rcf for 1 min to allow the particles to settle to attain a clear solution for the assay.

When performing the assay, the runs consisted of a blank, a reference solution and the sunscreen samples, adding the substrate solution each. GDB was used as a blank, while a prepared enzyme solution with a concentration of 0.02 mg/ml was used as the reference. For each run, 20 µl of the blank, reference and sunscreen samples were added to individual wells of a microtiter plate (96 well, Sarstedt 82.1581) in triplicates. Subsequently, 220 µl of the substrate solution was added in the row beneath the samples. The microtiter plate was then incubated for 10 min inside the microplate reader before 200 µl of the substrate solution was added to the samples in the rows above, thereby initiating the enzymatic reaction. The assay was started, plate reading was initiated, and enzyme activity was measured for 1 min.

Regarding Filter T5, a different approach was used due to its unique properties. Unlike other UV filters it did not separate when centrifuged and the samples were too concentrated when diluted to 1:5 to give comparable results. Multiple approaches were tested to measure the enzyme activity and the most effective approach was to dilute the sample with an additional factor of 10, resulting in a dilution ratio of 1:50. Moreover the duration for the assay was extended with a factor of 10 as well, resulting in 10 min duration instead of 1 min. Additionally, another blank was used, consisting of the final sunscreen sample without the enzyme.

## 3.3 Further Evaluations

Additional parameters were studied to gain a better understanding of the sunscreen's characteristics, including pH, absorbance and transmittance. The pH of each sample was measured by immersing pH test strips into the samples and estimating the corresponding pH value. The absorbance and transmittance were measured for the UV filters that had given the most promising previous results, namely Z1, Z2, T1, T2 and T5, using a UV-VIS spectrophotometer (Shimadzu, UV-1900i). These were measured for a spectrum within the wavelengths of 250-500 nm, as UVA and UVB stretches between 280-400 nm. To minimize

the absorbance of the UV wavelengths by the cuvette, a quartz cuvette was used for the measurements. The sunscreen samples were diluted in GDB to achieve an absorbance between 0-1 and they were run against a blank containing the base moisturizer diluted to the same dilution ratio as the samples. Filter Z1 and T1 were diluted to a ratio of 1:50 and Filter T5 was diluted to a ratio of 1:2000.

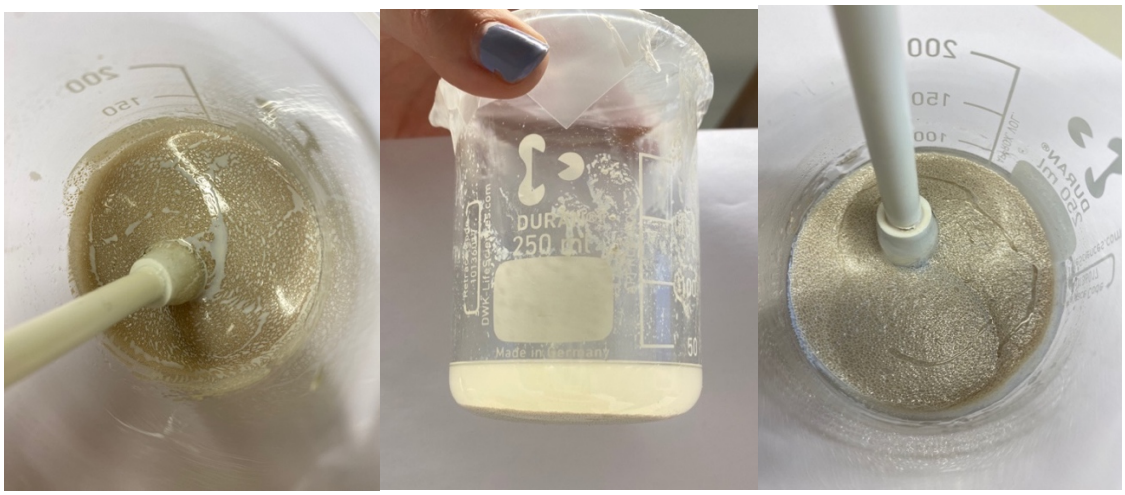
To determine which product to proceed with for future development, three sunscreens, containing three different UV filters (Filter Z1, T1 and T5) were evaluated according to customer satisfaction. Test samples of the different sunscreens were given to volunteers (n=26) accompanied by an evaluation form including both theoretical and practical questions. The responses collected from the form were compiled together with additional theoretical information to continue the development of the most favorable product.

# 4 Results

The formulation of various sunscreens containing different UV filters in combination with varying thickening agents yielded several results. The results comprise physical samples along with measurements of enzyme activity and stability for each sample, in addition to the absorbance and transmittance measurements. Ultimately, the results from the evaluation form created to address customer satisfaction regarding three different formulations have been compiled and are presented below.

## 4.1 Formulation

Consumers tend to choose cosmetic products based on their physical characteristics. Therefore, the initial experiments aimed at formulating sunscreens with an attractive appearance and pleasant feeling upon application to the skin. However, the first results, using Filter Z1 and Z2, were disappointing. They were not completely homogenous following to mixing and both displayed visible particles. They appeared as grey/beige in the color and possessed sticky sensation upon application to the skin. Furthermore, when left overnight, the gel structure had collapsed and phase separation had occurred, with a solid phase settling at the bottom and the liquid phase above, making them impossible to use, see Figure 1.



(A) (B) (C)

**Figure 1:** Sunscreens containing a combination of Filter Z1/Z2 and carbomer 1. (A) Sunscreen containing Filter Z2, (B) Sunscreen A after 24 h, (C) Sunscreen containing Filter Z1.

When replacing the carbomer with cellulose the formulation yielded notable improvements. It looked much smoother and maintained its consistency after storage, the color, however, remained grey/beige. The formulation with Filter Z1 can be seen in Figure 2, the formulation containing Filter Z2 looked the same.

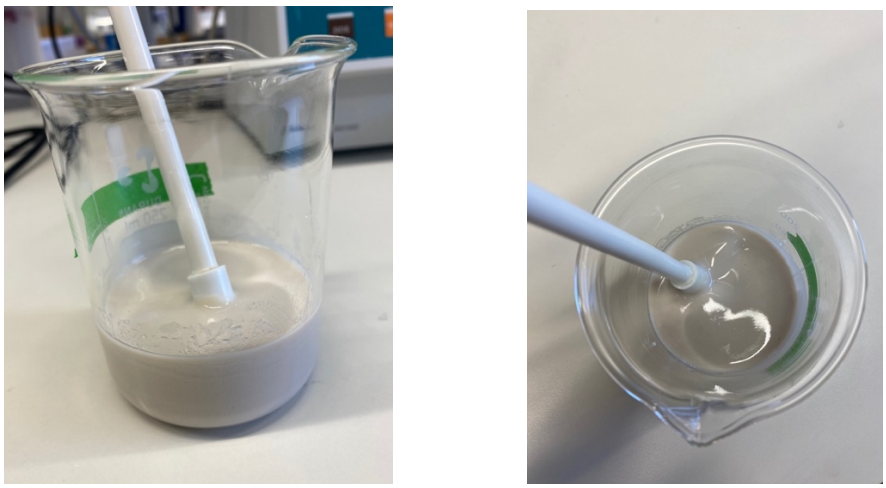
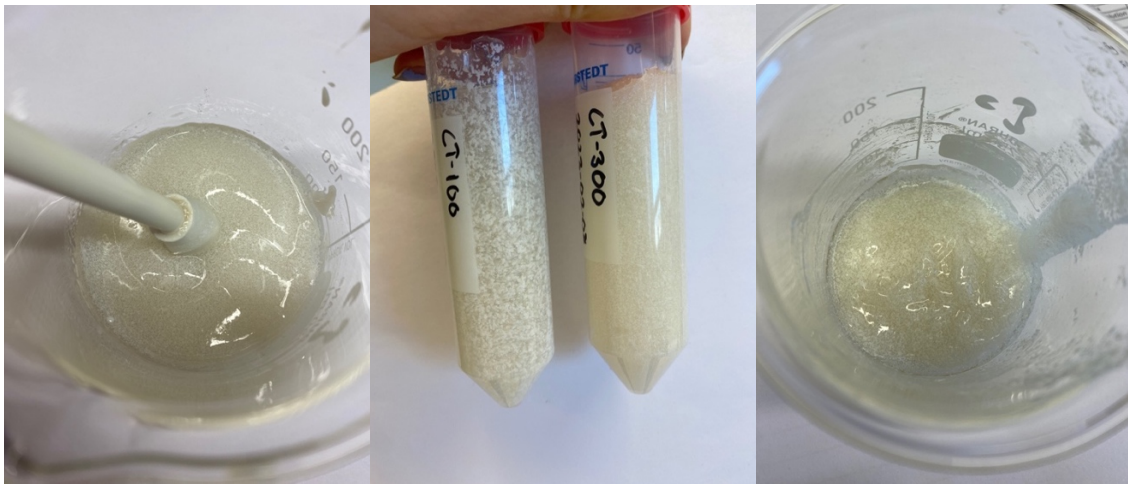


**Figure 2:** Sunscreen containing a combination of Filter Z1 and cellulose 1.

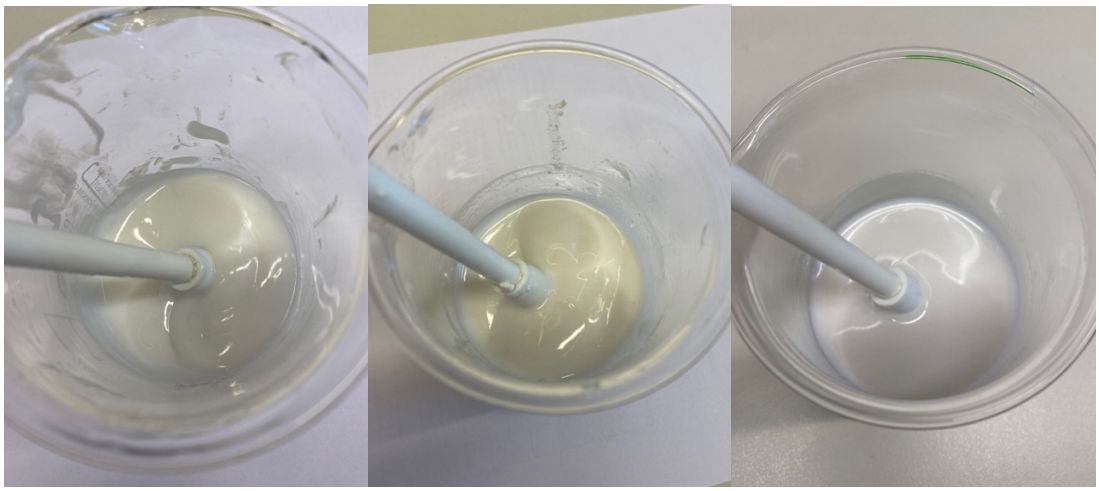
The sunscreens formulated with Filter T1 and T2, respectively, showed more promising results. They were more appealing to the eye as they were clearer, similar to the base moisturizer, however, they contained undesirable white visible particles, see Figure 3.




(A) (B) (C)

**Figure 3:** (A) Sunscreen containing Filter T1, (B) Comparison of Filter T1 and T2, (C) Sunscreen containing Filter T2.

Both sunscreens were able to maintain their characteristics during storage. Products, using Filter T1 and T2, with even better characteristics were obtained when components were mixed at a temperature of 70°C. The resulting products were white creams with a smooth texture, see Figure 4.

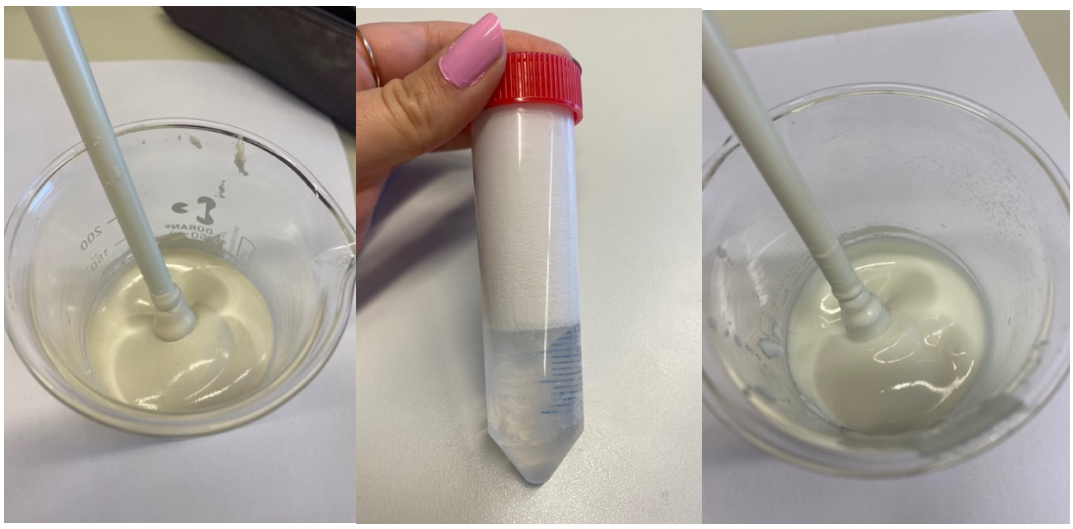


(A) (B) (C)

**Figure 4:** (A) Sunscreen containing Filter T1 and carbomer 1, (B) Sunscreen containing Filter T2 and carbomer 2, (C) Sunscreen containing Filter T1 and cellulose.

The three UV filters in the form of powders, namely Filter Z3, T3 and T4, resulted in smooth-looking off-white or white sunscreens, as shown in Figure 5. However, they differed from each other in terms of the sensation on the skin. Filter Z3 provided a smooth application, minimal white cast and low stickiness. It was absorbed into the skin more quickly than the

previously tested sunscreens. However, the formulation had a slightly dry appearance, suggesting incomplete mixing of the powder together with the other ingredients and when left for a week, it had also displayed phase separation. When cellulose was used instead of carbomer, the formulation remained intact, similar to the two other filters containing ZnO. On the other hand, the sunscreen with Filter T3 yielded an unpleasant skin sensation, despite its appealing visual appearance, upon application it resulted in noticeable particle texture on the skin resembling sand or peeling. The sunscreen left visible white particles on the skin and also felt sticky. The final filter tested in powder form, T4, demonstrated quite promising results as it was smooth, good spreadability and minimal stickiness, in fact, it was almost too dry in the sensation of the skin.



(A) (B) (C)

**Figure 5:** (A) Sunscreen containing Filter Z3 and carbomer 1, (B) Sunscreen containing Filter Z3 and carbomer 1 after one week, (C) Sunscreen containing Filter T3.

The final UV filter tested was Filter T5, which was tested in combination with several thickening agents. The following thickening agents were tested: carbomer 1, cellulose, xanthan gum and a combination of cellulose + xanthan gum. All formulations created with Filter T5 possessed a more intense white color compared with the previously tested UV filters, resulting in much more of a white cast when applied to the skin and it had to be worked into the skin more thoroughly. These formulations were more similar to commercial sunscreens rather than moisturizers containing sun protection, see Figure 6.

The initial formulation with carbomer 1 was not successful, it had an uneven consistency with several lumps, it was however considerably less sticky than previous formulations. The following formulations with cellulose and xanthan gum respectively were more preferable,

presenting a smoother consistency. However, these formulations were relatively runny and would need to be firmer. Another formulation was prepared with xanthan gum where the mixture was heated to 70°C while mixed, also this with disappointing results as the consistency became more lumpy and uneven along with still being very runny. The final formulation involving Filter T5 contained a combination of both cellulose and xanthan gum, resulting in a firmer consistency and favorable outcomes. It had a very smooth texture on the skin and was easy to apply, however, the intense white color of the formulation remained.

(A) (B) (C)

**Figure 6:** (A) Sunscreen containing Filter T5 and carbomer 1, (B) Sunscreen containing Filter T5 and carbomer 1 after one week, (C) Sunscreen containing Filter T5 and cellulose.

## 4.2 Enzyme Activity

The measurements for enzyme activity for all the formulated sunscreen samples are presented in Appendix A. The most relevant results are summarized in Figure 7 below. A representative image for the increase in absorbance in a chosen sample can also be seen in Figure 14 in Appendix A. Each measurement was performed in triplicate, and the mean value with the standard deviation was calculated. For each formulation, five measurements were performed over time including an initial measurement directly after formulation, two measurements after one week with samples stored at RT and 40°C, respectively, and two after 1-1.5 months also stored at RT and 40°C. The measured values were compared to the expected value for the enzyme activity for each initial sample. The expected value was based on the activity of 1 ml of the enzyme stock solution in 100 g of the sunscreen formulations and calculated to correspond to the amount of stock solution added to each sample. The percentage of the expected value achieved and the standard deviations are presented below.

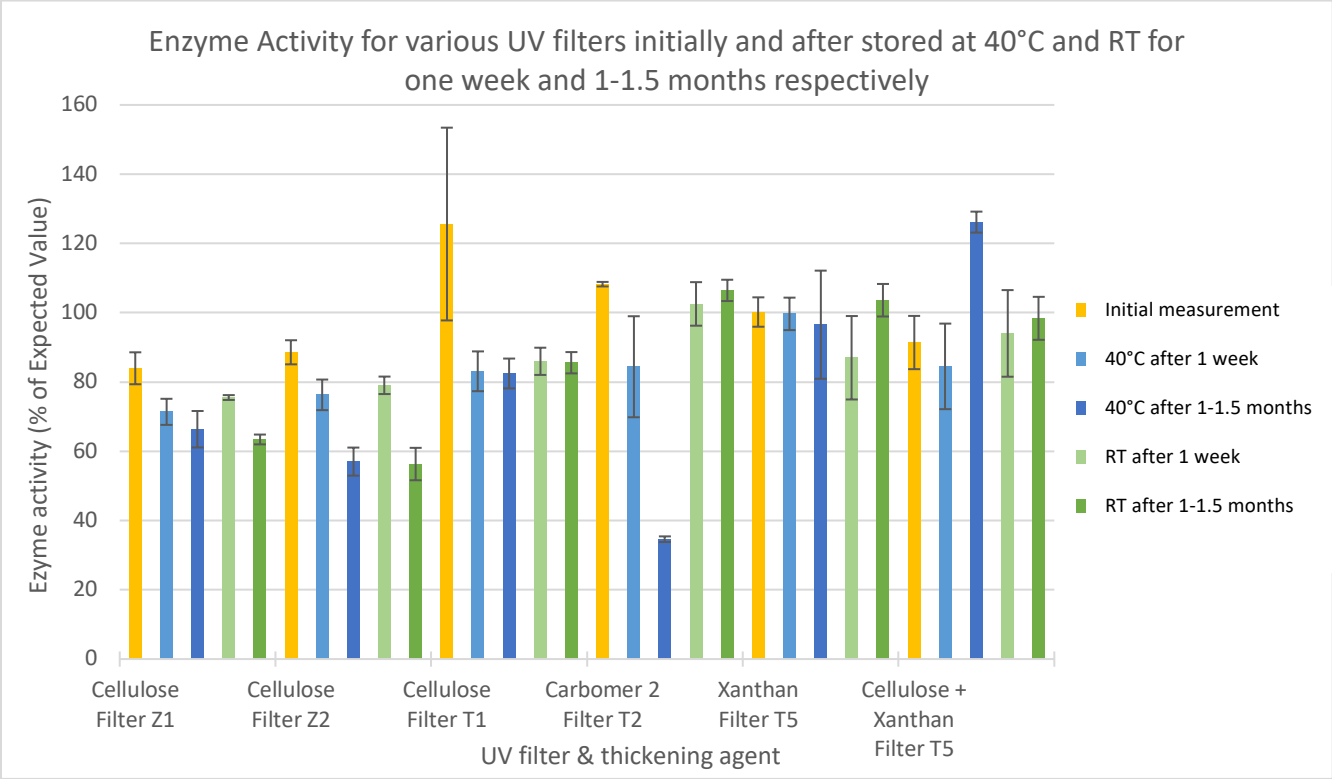



**Figure 7:** Percentage of the expected value for enzyme activity for various UV filters directly after formulation (yellow), after stored at 40°C (blue) in the dark and at RT (green) in daylight for one week and for 1-1.5 months respectively.

The yellow bars in Figure 7 represent the initial measurements taken immediately after the samples were formulated. The light blue bar, for every sample, correspond to enzyme activity measurements obtained after storing the samples in a dark environment at 40°C for one week and the dark blue bar corresponds to measurements taken after 1-1.5 months of storage under the same conditions. Similarly, the light green bar, for every sample, corresponds to measurements obtained after storing the samples in daylight at RT for one week, and the dark green bar corresponds to the measurements taken after 1-1.5 months of storage under the same conditions.

The enzyme appears to be active and stable in most of the formulations by observing the graph in figure 7. The initial enzyme activity for Filter Z1 and Z2, however, appears to be lower than expected and when compared to the other filters. Furthermore, the decrease in activity over time is larger for these two filters relative to the other filters, with the exception for Filter T2 where the activity for the sample stored at 40°C appears to have substantially decreased after

1-1.5 months. Although some measurements exceeded the expected value, their standard deviation could be an explanation of this.

## 4.3 Absorbance and Transmittance

The absorbance and transmittance of the most promising samples were measured. The absorbance measurements of Filter Z1, Z2 and T1, T2 did not show any significant peaks in absorbance within the UVA and UVB spectrum (280-400 nm), these results can be found in Appendix B. However, regarding Filter T5 in combination with different thickening agents, a distinct peak within these wavelengths is observed, see Figure 8. The three samples containing cellulose and xanthan gum all display a similar curve, in contrast to the sample containing carbomer 1 as this curve has a more distinct peak in the UV region.

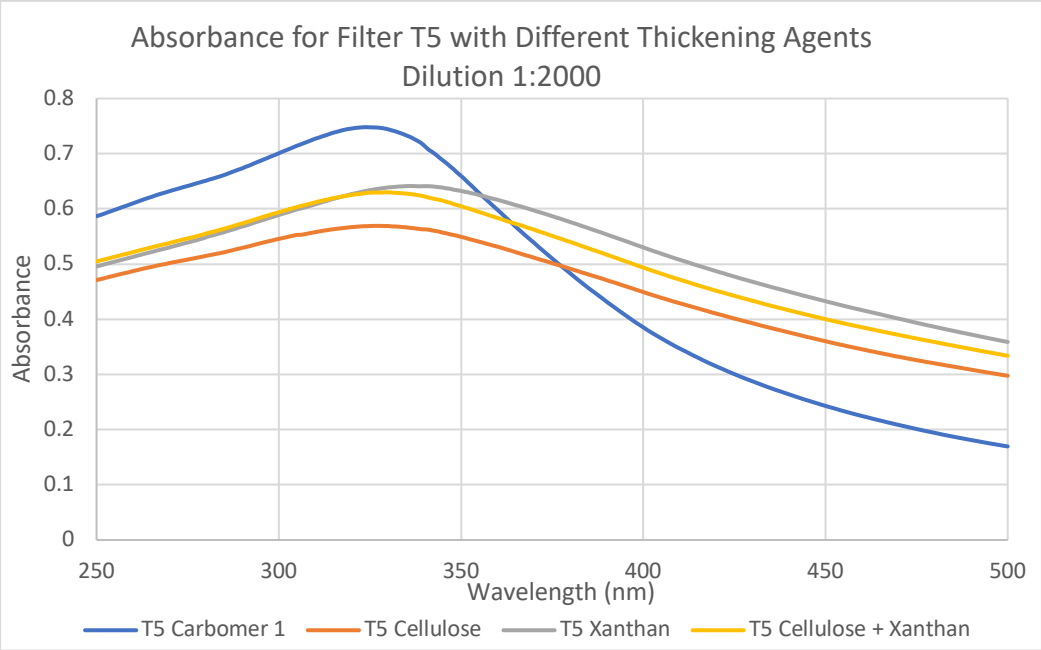



**Figure 8:** Absorbance spectrum for Filter T5 containing different thickening agents diluted 1:2000 in GDB with a blank containing the base moisturizer diluted 1:2000 in GDB.

Similar to the absorbance, the transmittance was measured to determine the extent of UV-light transmitted through the samples, hence the UV-light which is not absorbed or reflected. The transmittance for Filter Z1, Z2, T1 and T2 did not show any distinct peaks, see Appendix B, while in the case of Filter T5 the transmittance curve showed a similar trend to the absorbance curve but in the opposite direction. This implies that there was a decrease in transmittance at the same wavelengths where an increase in absorbance was observed, see Figure 9.

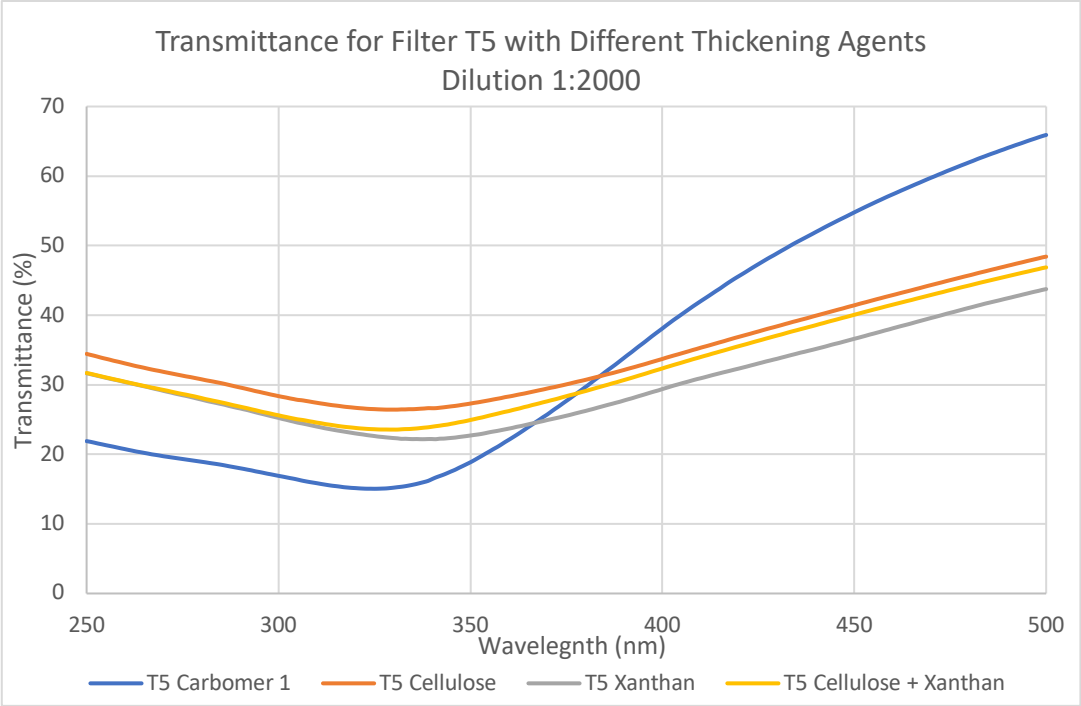



**Figure 9:** Absorbance spectrum for Filter T5 containing different thickening agents diluted 1:2000 in GDB with a blank containing the base moisturizer diluted 1:2000 in GDB.

The reflectance of the samples was also measured. However, this function of the spectrophotometer did not seem to be working correctly as these measurements gave identical results to those obtained for the transmittance, which is highly unlikely, therefore these measurements deemed unreliable and excluded from further analysis.

## 4.4 Further Evaluations

The pH values were determined using pH test strips presented in Figure 10 and Table 3. The majority of the formulations had a pH within the range of 5.5-7.5, except for the formulations containing a combination of ZnO and carbomer 1, which exhibited phase separation where the pH values were higher.

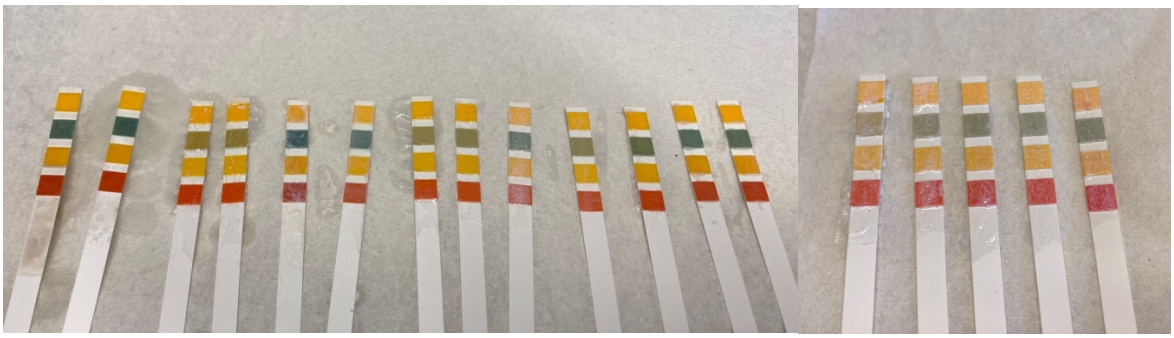


**Figure 10:** pH test strips for all formulations made, in the order from Table 3 from left to right.

**Table 3:** Estimated pH values for all formulations made.

| UV Filter | pH |
|---|---|
| Z1 + Carbomer 1 | 8 |
| Z2 + Carbomer 1 | 8 |
| T1 + Carbomer 1 | 5.5 |
| T2 + Carbomer 1 | 5.8 |
| Z3 + Carbomer 1 | 9 |
| T3 + Carbomer 1 | 7.5 |
| T1 + Carbomer 1 | 5.8 |
| T2 + Carbomer 2 | 5.8 |
| Z3 + Cellulose | 7 |
| T4 + Carbomer 1 | 6 |
| T1 + Cellulose | 7 |
| Z1 + Cellulose | 7.5 |
| Z2 + Cellulose | 7.5 |
| T5 + Carbomer 1 | 5.5 |
| T5 + Cellulose | 6 |
| T5 + Xanthan | 6 |
| T5 + Xanthan (heated) | 6 |
| T5 + Cellulose + Xanthan | 6 |

### 4.4.1 Customer Satisfaction

A cosmetic evaluation was performed where volunteers (n=26) participated in testing three different samples while answering both theoretical and practical questions regarding moisturizers containing sun protection in general and evaluating the three samples provided. The three samples were chosen as these contained UV filters with different characteristics and were the three most promising formulations. The samples were named Sample A, B and C and no specific information about the different samples and UV filters incorporated was provided. The only information provided was a general ingredient list without assessing the ingredients to a specific sample. Which sample that corresponds to which UV filter can be seen in Table 4 below.

**Table 4:** Samples corresponding to sunscreen formulations.

| Sample name | UV filter & Thickening Agent |
|---|---|

| | |
|---|---|
| Sample A | Filter T1 + Cellulose |
| Sample B | Filter Z1 + Cellulose |
| Sample C | Filter T5 + Cellulose + Xanthan gum |

The results from the evaluation, and the evaluation form itself, can be found in Appendix C, while the most relevant results are compiled below. The volunteers participating in the sample testing were asked what SPF they would prefer in a facial moisturizer with sun protection and 50% answered SPF 30, 30.8% answered SPF 40+ and 19.2% answered SPF 15. The participants were also asked to rank the importance of various factors when purchasing this type of sunscreen, and the average ranking for each parameter was calculated. The results from this ranking can be seen in Figure 11, where 1 is considered the least important and 6 is the most important.

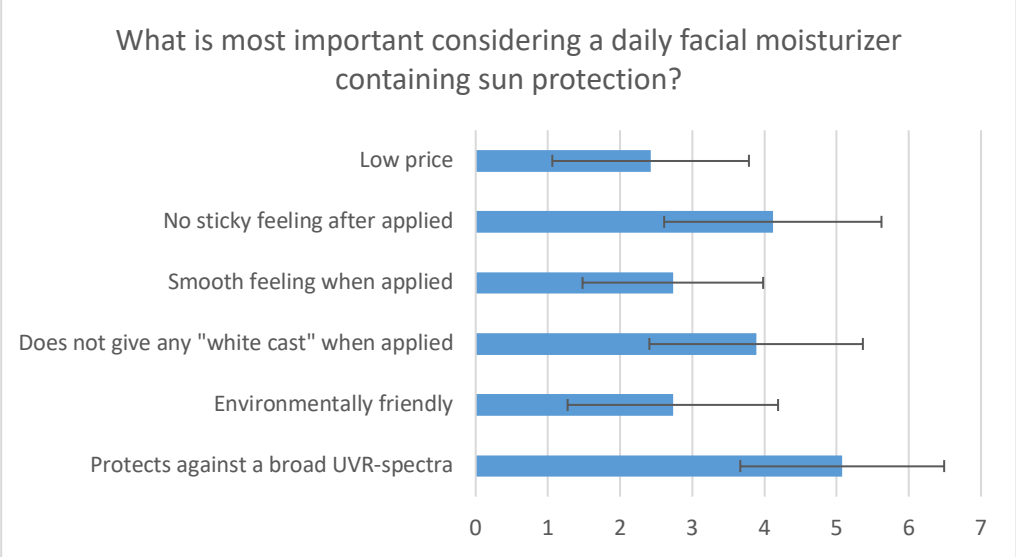



**Figure 11:** Calculated average value and standard deviation for parameters considered most and least important when buying a facial moisturizer containing sun protection (n=26).

Following the theoretical questions, the volunteers were instructed to test the three different samples and evaluate nine different parameters considering both appearance and sensation. The average ranking for each parameter was calculated for each sample and the results are presented below in Figure 12. The parameters were ranked on a scale from 1-5, with 5 indicating the highest ranking.

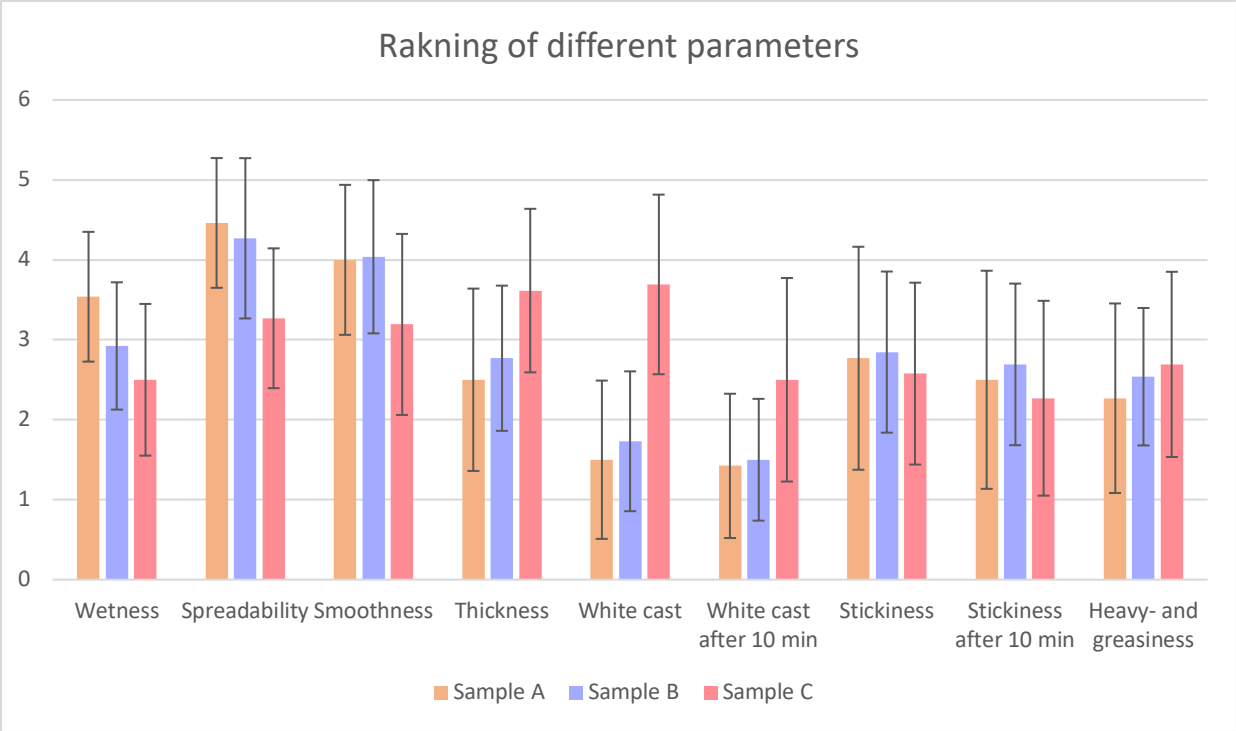



**Figure 12:** Calculated average value and standard deviation for parameters ranked when testing three different sunscreen samples.

In the final question the participants were asked to choose which sample they preferred and would purchase, and to rank the remaining two in order of preference. The results in Figure 13 indicate the percentage of participants who would choose each sample respectively.

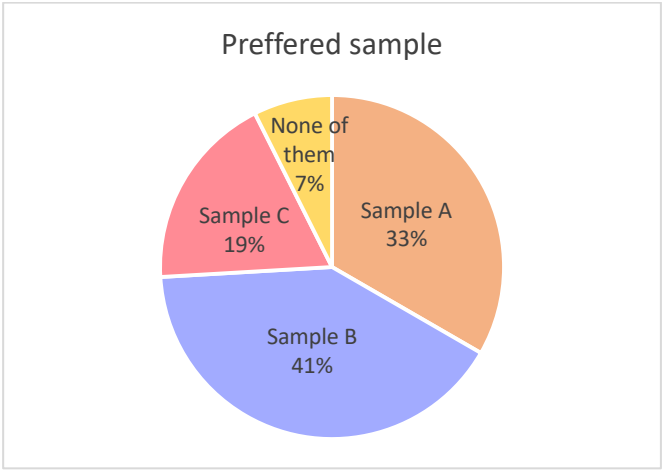



**Figure 13:** Percentage of participants that would buy a certain sample (n=26).

Additional questions regarding participants' age, gender and frequency of use of a moisturizer containing sun protection were included, but further discussion of these topics will not be presented in this report.

# 5 Discussion

Since the ultimate goal is to put this product on the market several factors must be taken into account to ensure successful product development. The enzyme must exhibit sufficient stability over a defined period of time, while the formulation must maintain its characteristics and avoid phase separation. Additionally, to achieve its intended function as a sunscreen, the product must provide effective and persistent protection against UVR. Finally, to enhance customer satisfaction, the product should possess desirable sensory attributes.

## 5.1 Formulation

The incorporation of the different UV filters into the base moisturizer made a great difference to each formulation, despite the fact that only one ingredient was added. The moisturizer is a visually transparent, aesthetically pleasing product with a desirable sensory profile upon application. Upon introduction of the UV filters, all formulations acquired a white/beige color, resulting in a product appearance more similar to other moisturizers/creams and conventional sunscreens.

The formulations containing the ZnO based filters, Z1, Z2 and Z3, in combination with carbomer 1 were incompatible with the base moisturizer. Their incorporation caused phase separation and a collapse of the gel structure within 48 hours. This phenomenon likely arose from the interaction between the carboxylic groups of the carbomer and the free $Zn^{2+}$ ions derived from the ZnO filters. Given that $Zn^{2+}$ is a divalent ion, it can react with carboxylic groups of the carbomer and form insoluble metal complexes, ultimately causing carbomer collapse. Replacing the carbomer with cellulose or xanthan gum avoided this issue, thereby preserving the structure of the formulations containing ZnO. The sunscreens containing $TiO_2$ in combination with the carbomers, however, appeared stable under storage conditions. Nevertheless, it is worthwhile to consider alternative thickening agents to carbomer, such as cellulose or xanthan gum, as the carbocyclic groups of the carbomer can react with salts, thereby diminishing gel stability also in the $TiO_2$ formulations. Furthermore, given that the skin naturally produces sweat containing salt, the choice of thickening agent may impact formulation stability when exposed to the skin.

The combination of Filter T1 and T2 with the other ingredients was in need of a water bath at 70°C to obtain a homogenous mixture, whereas Filter T3, T4 and T5 did not require this step. The differential behavior is likely attributed to the composition of the oil based $TiO_2$

filters. These filters are typically hydrophobic, making them more difficult to mix with water. By introducing heat, the solubility of certain substances is improved. Moreover, a temperature caused decrease in viscosity likely allows better distribution and dispersion of substances between the oil and water providing a more homogenous mixture. Alternatively, the non-heated mixture may require longer and more intensive mixing/homogenization to achieve a homogenous mixture.

There were no significant challenges encountered with the remaining formulations (with Filter T3, T4 and T5), they were all easily combined into a homogenous mixture and presented quite desirable sensory and visual characteristics. Some were more preferable than others from a sensory perspective, resulting in the exclusion of certain formulations from further development. Although these excluded formulations may have potential improvements, the formulations showing more promising results were prioritized for further development based on their superior performance.

## 5.2 Enzyme activity

The measurements of the enzyme activity over time provided valuable insight of the enzyme stability and how the activity is affected during storage. Given that the ultimate product is intended as a daily facial moisturizer, it may remain on store shelves or in costumers' bathroom cabinets for extended periods. Therefore, long-term measurements and monitoring of the enzyme stability is necessary to ensure enzyme activity is maintained upon storage.

Analysis of the enzyme activity data, presented in Figure 7 in the results, shows that initial activity levels, directly after formulation, varied among the different UV filters. Upon comparing the measured activity with the anticipated value, calculated based on the amount of enzyme added, some of the UV filters display a lower activity level than expected, approximately 80-90%, while others even had higher, over 100%. The lower activity in the formulations with Filter Z1 and Z2 may be a result of absorption of a fraction of the enzyme by the UV filters. Interestingly, a somewhat lower enzyme activity was seen in the UV filters, free from coating (Filter Z1 and Z2). This observation suggests a potential increase in absorption levels within these UV filters, and that the particle coating may decrease this risk of absorption. Another consequence of the absorption could be that in the samples that were centrifuged prior to enzyme activity measurements (all filters except Filter T5), the pellet may contain enzyme molecules that have been absorbed by the UV filter, and therefore will not be present in the enzyme activity measurement resulting in a decrease in activity.

Another explanation could be that certain UV filters, or any ingredient within the commercial UV filter formulation, interact directly with the enzymes, as enzymes have high sensitivity towards both the environment and other chemicals/ingredients. Such interactions could lead to enzyme inhibition and a decrease in activity toward the substrate. Alternatively, measurement errors may have contributed to the variety in enzyme activity. Prior to measurement, several samples underwent centrifugation, which could have resulted in uneven sampling of both the UV filters and the triplicates of each sample. This may, in turn, explain the large standard deviations observed in certain measured values, for instance the initial measurement for Filter T1 and the measurement after one week for Filter T2. This could explain why Filter T1 appears to have exceeded 100% of the initially expected value. The difference in initial enzyme activity levels between the various UV filters, specifically between Filter T5 and the others, may also be due to differences in the preparative process prior to measurement. As the samples containing Filter T5 were significantly more concentrated, in regards to the UV filter, the samples were diluted 10 times more and measured 10 times longer in the assay. Furthermore, the samples containing Filter T5 were not centrifuged which could have influenced the results and makes comparison with the less diluted samples more challenging. As the formulations containing Filter T5 exhibited promising outcomes in terms of enzyme stability, this may also suggest that this monitoring approach might be more efficient compared to the protocol used for the other formulations. However, it might not be possible to use this approach for the previous formulations due to their difference in composition.

After one week of storage, certain UV filters exhibited a high level of loss of enzyme activity, indicating low enzyme stability and these filters could be ruled out from further development, (Filter T3 and T4, see Appendix A). Nevertheless, several formulations showed enzyme activity levels that were close to the initial measurement both after one week, and after 1-1.5 months, suggesting good initial enzyme stability. Based on the measured activity, it can be concluded that Filter T5 gives the most stable formulations. Furthermore, disregarding the initial measurement for Filter T1, the enzyme activity after storage for this formulation also shows very promising results. Taking into account the large standard deviation from the initial measurement, it is likely that the enzyme activity value for Filter T1 is lower than 125%, indicating a reasonably favorable long-term stability. The activity decreased over time for all formulations, and the decrease in activity was slightly greater for the samples stored at 40°C compared to those stored at RT for most of the samples, see Figure 7. This observation can be explained by the fact that enzymes are sensitive to changes in temperature. The difference

might not be significant considering a temperature increase of 15 to 20°C would suggest a 3-to-4-fold decrease in stability. The temperature change may alter the enzyme's structure or even accelerate degradation of the enzyme decreasing the activity.

In order to thoroughly assess the long-term stability of all products formulated, it is necessary to conduct additional measurements over an extended period of time. The long-term stability assessment ensures both preservation of enzyme activity but also the stability of the formulation, including factors such as the absence of phase separation. The aim for the product is to maintain a shelf-life activity that is stable for 30 months, thereby allowing for a 12-month stability claim once the product is opened. To accomplish this, accelerated stability testing can be performed, in this case involving a nine-month testing period with samples stored at 40°C. This approach includes exaggerated storage conditions to allow for the calculation of the product's stability over a 30-month period. This type of accelerated stability testing was not possible in this project due to lack of time, however it would be of great interest for further development of the products.

## 5.3 Absorbance and Transmittance

Due to the mechanism of action of inorganic UV filters, including both absorbing and reflecting the UVR, it is important to measure and compare both of these factors along with the transmittance for the tested UV filters. By adding up the absorbance, transmittance and the reflection, and converting the data into the same units, this should account for all the UVR emitted. The absorbance was measured for Filter Z1, Z2, T1, T2 and T5. However, for Filter Z1, Z2, T1 and T2, the absorbance spectra did not show any peaks within the UVR wavelengths of interest which would indicate increased absorbance for UVA and UVB, as presented in Appendix B. This could be attributed to the ineffectiveness of these filters in providing adequate protection against UVR, however this is probably not the case. It could also be a result of some error in the spectrophotometer and/or in the experimental setup which could affect the accuracy of the measurements. In contrast, the absorbance spectrum for Filter T5 demonstrated a clear peak within the UVR wavelength, as shown in Figure 8, indicating good protection against both UVA and UVB. Furthermore, the formulation containing a combination of T5 and carbomer 1 exhibited a more defined peak and even higher absorbance for UVR compared to other thickening agents. This suggests the potential for improved UVR protection either in a combination with the carbomer and the different UV filters or that the carbomer itself may absorb a portion of the UVR which the cellulose does not. Since the carbomer contains

carboxylic acids which can absorb some UVR this is most likely the explanation for this case. Comparing the efficacy of the different UV filters investigated is challenging due to the limited availability of favorable results. A comprehensive analysis of the peak size and the wavelength range covered by each filter would be advantageous in determining which UV filter would protect most efficiently against both UVA and UVB.

Transmittance is a measurement of the amount of light that passes through a sample. Therefore, depending on the reflection, it is expected that the curve for the transmittance is in principle the opposite of the absorbance curve. This as the light that is not absorbed or reflected is instead transmitted through the sample. This trend was observed for all measurements performed and can be observed in Figure 9 and Appendix B. However, in the case of these inorganic UV filters, the transmittance should not be a complete inverse of the absorbance, as some UV light may be reflected as well. As the spectrophotometer used in this study was unable to measure reflectance, no results for this parameter were obtained, this may also explain why the absorbance and the transmittance are each other's opposite since the spectrophotometer did not consider the reflection. The measurements of the reflectance spectra would be of great interest for determining the extent of the mechanism of action of the UV filters and allow for a comparison of their reflectance and absorbance, which are the two primary mode of actions expected. However, to ensure accurate SPF values in the final products, absorbance measurements must be conducted by an independent accredited laboratory.

To draw any conclusions regarding the effectiveness of the filters in the long-term, additional measurements over a prolonged period of time are required, longer than was possible for this project. As the formulations are stored, their photostability may decrease, which in turn decreases the level of protection against UVR, especially if the formulations are exposed to sunlight and stored in a container that does not provide protection against light. This highlights the need of proper packaging design for the final products.

## 5.4 Further Evaluations

### 5.4.1 pH

The measured pH of the formulated products fell within the range of 5.5-7.5, except for the formulations containing the combination of ZnO and carbomer 1, where the pH was higher, around pH 8.0-9.0. This could be a result of the interaction between the $Zn^{+}$ and the carboxylic acid present in the carbomer, leading to a shift in pH. An explanation for this is that the carboxylic acid typically contributes to lowering the pH, while ZnO acts as a base, thereby

increasing the pH. This pH increase could be avoided by adjusting the pH through addition of more acid, which also may reduce the risk of gel collapse.

It is important to maintain the pH within a specific range, both since enzymes can be dependent on their environment, whereby pH and temperature may affect their activity. Additionally, aligning the formulations pH closely with the natural pH of the skin is important to avoid damage of the natural balance of the skin. The formulations containing ZnO in combination with cellulose demonstrated pH values around 7.0-7.5 (Filter Z1 and Z2), while those containing $TiO_2$ had lower pH values (Filter T1, T2 and T5). This is probably also due to the slightly alkaline nature of ZnO, increasing the pH in these formulations. The variations observed in the initial measurements of the enzyme activity are, however, unlikely to be influenced solely by the pH, as the protease assay incorporates a buffer system maintaining consistent pH in all samples during the measurements. However, the difference in the enzyme stability could potentially be a result of the varying pH values. Moreover, by addition of a stronger buffer into these formulations, the pH can be maintained at a level ideal for both enzyme and the cosmetic function of the sunscreen.

### 5.4.2 Customer Satisfaction

The results from the cosmetic evaluation provide information about both theoretical and practical preferences related to a moisturizer containing sun protection. It should be noted that factors such as age, gender and geographical location may influence preferences and responses of the participants. However, these factors have not been taken into account for the discussion of the results in this work. Studying the results, it is clear that the majority of the participants want an SPF of 30 in a daily facial moisturizer. When asked about the relative importance of various factors associated with the sunscreen, Figure 11 reveals that “Protects against a broad UVR-spectra” ranks the highest in terms of significance, followed closely by “No sticky feeling when applied” while the aspect of “Low price” appears to be the least important. Considering that one of the primary objectives for this product is to protect against a broad UVR spectra, the fact that this aspect also is considered the most important factor to the customers is encouraging. It is, however, important to consider the quite large standard deviations in figure 11, indicating the difference in preferences between the participants.

When compiling the results of the different parameters evaluated regarding the three different samples tested some difference can be observed, see Figure 12. Sample A corresponds to Filter T1, Sample B to Filter Z1 and Sample C to Filter T5. All three samples received similar

ranking considering stickiness and heavy- and greasiness, respectively, where they are ranked around 2.5 out of 5 indicating a moderate stickiness level. This might raise concern as the absence of a "sticky feeling when applied" was considered highly important and should therefore be addressed in future formulations. Moreover, the spreadability and smoothness is ranked higher for Sample A and B compared to Sample C, making them more favorable in terms of formulation, while Sample C presents significantly higher levels of white cast, also indicating higher preference for Sample A and B. Furthermore, the standard deviations are quite large for these parameters as well, indicating varying preferences among the participants which also must be taken into consideration. This means that any of the formulations would be appropriate for a certain customer.

Regarding which sample the participants preferred and would actually purchase, Sample B is the most favored sample, see Figure 13. Approximately 41% of the participants indicated sample B as their favorite sample, followed by 33% choosing Sample A, while only 19% chose Sample C and 7% expressed no interest in purchasing any of the products. Notably, Sample B is the only sample containing ZnO, which offers slightly better protection against both UVA and UVB compared to $TiO_2$. Since this was the most important factor according to the participants, Sample B, which corresponds to Filter Z1, emerges as the top candidate within the different formulation.

# 6 Conclusion

In conclusion, to develop a successful enzyme-based sunscreen there are several factors to take into consideration. Firstly, the formulation itself must exhibit long-term stability without any indication of phase separation. Additionally, the enzyme must also retain activity and stability over an extended duration to ensure effective exfoliating properties mediated by the proteases. Another important aspect for this type of product is to ensure an effective and persistent protection against a broad spectrum of UVR, in this case indicating both UVA and UVB wavelengths. Simultaneously, it is essential to achieve desirable sensory attributes and characteristics for customer satisfaction.

Formulations containing a combination of ZnO and carbomer were incompatible as they displayed phase separation. By replacing the carbomer with cellulose this issue was resolved. Cellulose was found to be the optimal alternative for the remaining UV filters containing $TiO_2$ as well, making it the preferred thickening agent to proceed with for further development. The enzymatic activity showed slight variations between the different UV filters, potentially

influenced by interactions with the enzyme, absorption of the enzyme by the UV filters, or due to measurement errors. Notably, Filter T5 exhibited the most promising enzyme stability, however, several additional tests over an extended period of time is required, along with more measurements of the absorbance and reflectance of the various filters, to draw any definitive conclusions about the most promising formulation.

Considering customer preferences, Filter Z1 emerged as the most desirable product in terms of both theoretical and practical aspects, making this an intriguing candidate for further development. Filter Z1 also has a greater particle size indicating lower negative impact on both the environment and human health. Regarding the regulatory aspects, Filter Z1 is in compliance with the regulatory frameworks in EU, USA and China, along with numerous other countries. This enables the market placement of the product on a global scale. Overall, Filter Z1, T1 and T5 in combination with cellulose all gave promising results in multiple aspects including formulation characteristics, enzyme activity and stability and customer satisfaction. However, further evaluations are needed to finalize the development of this product and to prepare it for market introduction.

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# 8 Appendix

## A. Results from Protease Assay

**Table 5:** Percentage of the expected value for enzyme activity for various UV filters directly after formulation and after stored at 40°C in the dark and at RT in daylight respectively for one week.

| UV Filter | Thickener | Date | % of Expected Value | Standard Deviation (%) | Coefficient Variation (%) | Comment |
|---|---|---|---|---|---|---|
| Z1 | Carbomer 1 | 2023-02-07 | 39.41 | 3.16 | 8.03 | Initial |
| | | 2023-02-14 | 84.05 | 2.08 | 2.47 | 40°C |
| | | | 83.52 | 3.47 | 4.16 | RT |
| | Cellulose | 2023-03-03 | 83.96 | 4.61 | 5.49 | Initial |
| | | 2023-03-17 | 71.39 | 3.77 | 5.28 | 40°C |
| | | 2023-04-18 | 66.36 | 5.26 | 7.93 | |
| | | 2023-03-10 | 75.52 | 0.70 | 0.93 | RT |
| | | 2023-04-18 | 63.41 | 1.42 | 2.23 | |
| Z2 | Carbomer 1 | 2023-02-03 | 137.17 | 18.83 | 13.73 | Initial |
| | | 2023-02-10 | 114.69 | 6.18 | 5.39 | 40°C |
| | | | 105.43 | 8.50 | 8.06 | RT |
| | Cellulose | 2023-03-03 | 88.58 | 3.48 | 3.93 | Initial |
| | | 2023-03-17 | 76.29 | 4.42 | 5.79 | 40°C |
| | | 2023-04-18 | 57.01 | 4.05 | 7.11 | |
| | | 2023-03-10 | 79.04 | 2.52 | 3.19 | RT |
| | | 2023-04-18 | 56.31 | 4.68 | 8.31 | |
| T1 | Carbomer 1 | 2023-02-07 | 102.97 | 6.66 | 6.47 | Initial |
| | | 2023-02-14 | 93.44 | 3.43 | 3.67 | 40°C |
| | | | 87.36 | 2.08 | 2.39 | RT |
| | | 2023-02-21 | 103.15 | 6.38 | 6.19 | Initial (heated) |
| | | 2023-02-28 | 92.51 | 3.82 | 4.13 | 40°C |
| | | | 82.94 | 2.15 | 2.59 | RT |
| | Cellulose 2% | 2023-02-27 | 75.48 | 1.31 | 1.73 | Incorrect |
| | | | 125.61 | 27.83 | 22.16 | Initial (heated) |
| | | 2023-03-06 | 83.09 | 5.75 | 6.92 | 40°C |
| | | 2023-04-18 | 82.46 | 4.31 | 5.22 | |
| | | 2023-03-06 | 85.97 | 3.93 | 4.57 | RT |
| | | 2023-04-18 | 85.56 | 3.07 | 3.59 | |
| T2 | Carbomer 1 | 2023-02-08 | 137.06 | 2.75 | 2.01 | Incorrect |
| | | 2023-02-09 | 101.55 | 8.86 | 8.72 | Initial |
| | | 2023-02-15 | 115.28 | 1.16 | 1.01 | 40°C |
| | | | 109.39 | 5.31 | 4.85 | RT |
| | Carbomer 2 | 2023-02-22 | 108.24 | 0.66 | 0.61 | Initial (heated) |

| | | | | | | |
|---|---|---|---|---|---|---|
| | | 2023-03-01 | 84.40 | 14.59 | 17.28 | 40°C |
| | | 2023-04-18 | 34.61 | 0.82 | 2.35 | |
| | | 2023-03-01 | 102.54 | 6.29 | 6.13 | RT |
| | | 2023-04-18 | 106.45 | 3.08 | 2.89 | |
| Z3 | Carbomer 1 | 2023-02-08 | 33.15 | 1.09 | 3.30 | Incorrect |
| | | 2023-02-09 | 41.25 | 14.71 | 35.66 | Initial |
| | | 2023-02-15 | 92.86 | 49.63 | 53.45 | 40°C |
| | | | 59.04 | 25.90 | 43.88 | RT |
| | Cellulose 3% | 2023-02-24 | 118.92 | 31.34 | 26.35 | Initial |
| | | 2023-03-03 | 79.44 | 5.22 | 6.57 | 40°C |
| | | | 106.61 | 6.230 | 5.91 | RT |
| T3 | Carbomer 1 | 2023-02-09 | 65.75 | 9.62 | 14.63 | Initial |
| | | 2023-02-16 | 37.24 | 5.52 | 14.82 | 40°C |
| | | | 55.89 | 1.39 | 2.49 | RT |
| | | | 40.39 | 3.29 | 8.13 | 40°C (New) |
| | | | 61.23 | 6.80 | 11.11 | RT (New) |
| T4 | Carbomer 1 | 2023-02-24 | 76.07 | 7.81 | 10.27 | Initial |
| | | 2023-03-03 | 1.56 | 2.65 | 169.35 | 40°C |
| | | | 51.71 | 2.00 | 3.88 | RT |
| | | | 1.62 | 2.23 | 138.02 | 40°C (New) |
| | | | 56.02 | 4.64 | 8.29 | RT (New) |
| T5 | Carbomer 1 | 2023-03-09 | NaN | NaN | NaN | Incorrect |
| | | | 314.52 | 2.76 | 0.88 | Initial |
| | | Mix T5/ref | 59.96 | 6.50 | 10.85 | Mix of 10 µL of T5 and 10 of ref |
| | | | 53.98 | 5.18 | 9.59 | |
| | | 2023-03-17 | 43.27 | 12.40 | 28.65 | |
| | | | 50.06 | 27.19 | 54.33 | |
| | Cellulose | 2023-03-10 | 297.00 | 66.21 | 22.29 | Initial |
| | | 2023-03-17 | 92.09 | 10.91 | 11.85 | 40°C |
| | | | 92.34 | 3.51 | 3.80 | RT |
| | Xanthan | 2023-03-14 | 100.20 | 4.24 | 4.24 | Initial |
| | | 2023-03-21 | 99.67 | 4.69 | 4.70 | 40°C |
| | | 2023-04-18 | 96.54 | 15.61 | 16.17 | |
| | | 2023-03-21 | 87.00 | 12.05 | 13.85 | RT |
| | | 2023-04-18 | 103.61 | 4.70 | 4.53 | |
| | | 2023-03-15 | 105.36 | 6.66 | 6.32 | Initial (heated) |
| | | 2023-03-22 | 105.92 | 8.78 | 8.29 | 40°C |
| | | | 164.27 | 93.78 | 57.09 | RT |
| | Cellulose + Xanthan | 2023-03-16 | 91.39 | 7.69 | 8.41 | Initial |
| | | 2023-03-23 | 84.51 | 12.35 | 14.63 | 40°C |
| | | 2023-04-18 | 126.16 | 3.03 | 2.40 | |
| | | 2023-03-23 | 94.03 | 12.51 | 13.30 | RT |

| | | | | | | |
|---|---|---|---|---|---|---|
| | | 2023-04-18 | 98.38 | 6.21 | 6.31 | |

**Table 6:** Percentage of the expected value for enzyme activity for various UV filters after stored at 40°C in the dark and at RT in daylight respectively for 1-1.5 months.

| UV filter | Thickener | Date | % of Expected Value | Standard Deviation (%) | Coefficient Variation (%) | Comment |
|---|---|---|---|---|---|---|
| T1 | Cellulose | 2023-04-18 | 82.46 | 4.30 | 5.22 | 40°C |
| | | 2023-04-18 | 85.56 | 3.07 | 3.59 | RT |
| T2 | | 2023-04-18 | 34.61 | 0.81 | 2.35 | 40°C |
| | | 2023-04-18 | 106.45 | 3.08 | 2.89 | RT |
| Z1 | Cellulose | 2023-04-18 | 75.70 | 10.34 | 13.66 | 40°C |
| | | 2023-04-18 | 74.40 | 4.48 | 6.02 | RT |
| Z2 | | 2023-04-18 | 61.32 | 15.12 | 24.66 | 40°C |
| | | 2023-04-18 | 73.69 | 27.11 | 36.79 | RT |
| Z1 | Cellulose | 2023-04-18 | 66.36 | 5.26 | 7.93 | 40°C |
| | | 2023-04-18 | 63.41 | 1.42 | 2.23 | RT |
| Z2 | | 2023-04-18 | 57.01 | 4.05 | 7.11 | 40°C |
| | | 2023-04-18 | 56.31 | 4.68 | 8.31 | RT |
| T5 | Cellulose + Xanthan | 2023-04-18 | 126.16 | 3.03 | 2.40 | 40°C |
| | | 2023-04-18 | 98.38 | 6.21 | 6.31 | RT |
| | Cellulose | 2023-04-18 | 96.54 | 15.61 | 16.17 | 40°C |
| | | 2023-04-18 | 103.61 | 4.70 | 4.53 | RT |

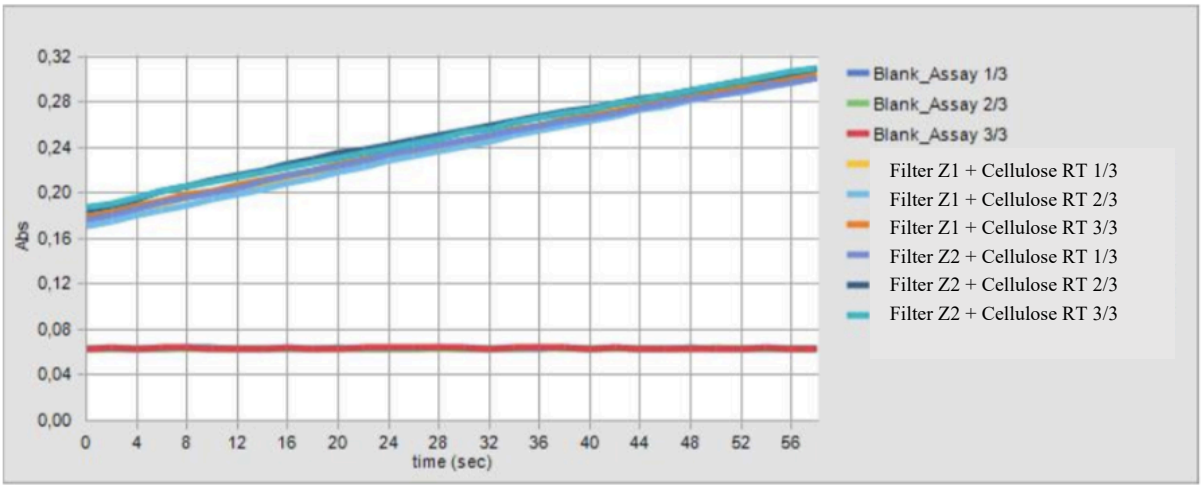


**Figure 14:** Representative image measuring the enzyme activity for a sample. Increase of absorbance over time during a protease assay run.

## B. Absorbance and Transmittance spectrum

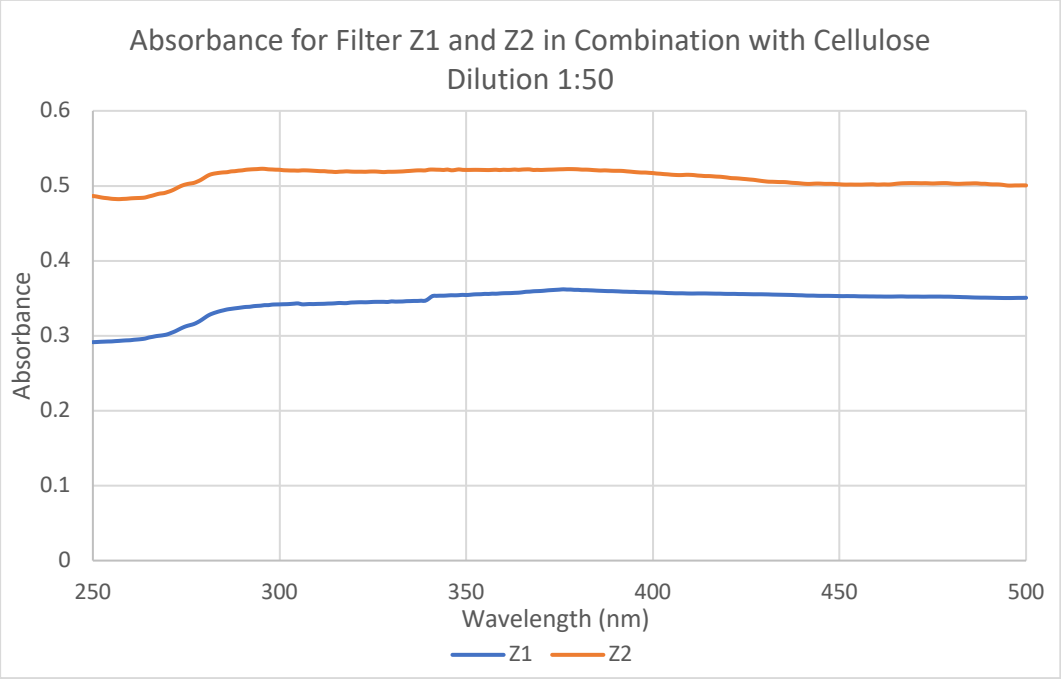


**Figure 15:** Absorbance spectrum for Filter Z1 and Z2 in combination with cellulose, diluted 1:50 in GDB with a blank containing the base moisturizer diluted 1:50 in GDB.

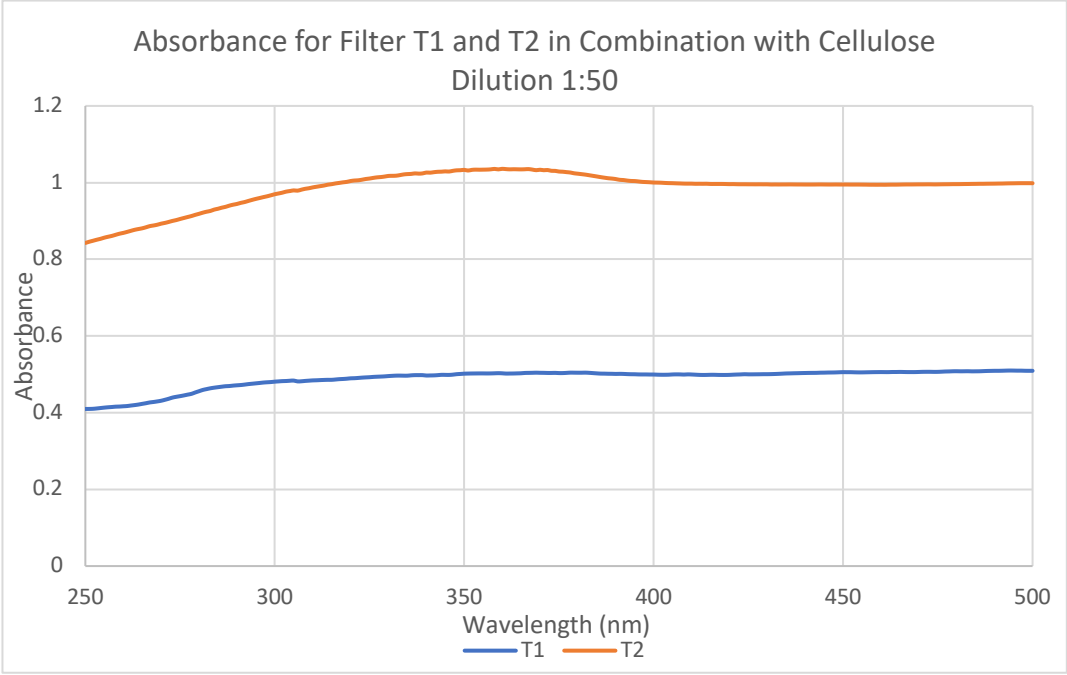


**Figure 16:** Absorbance spectrum for Filter T1 and T2 in combination with cellulose, diluted 1:50 in GDB with a blank containing the base moisturizer diluted 1:50 in GDB.

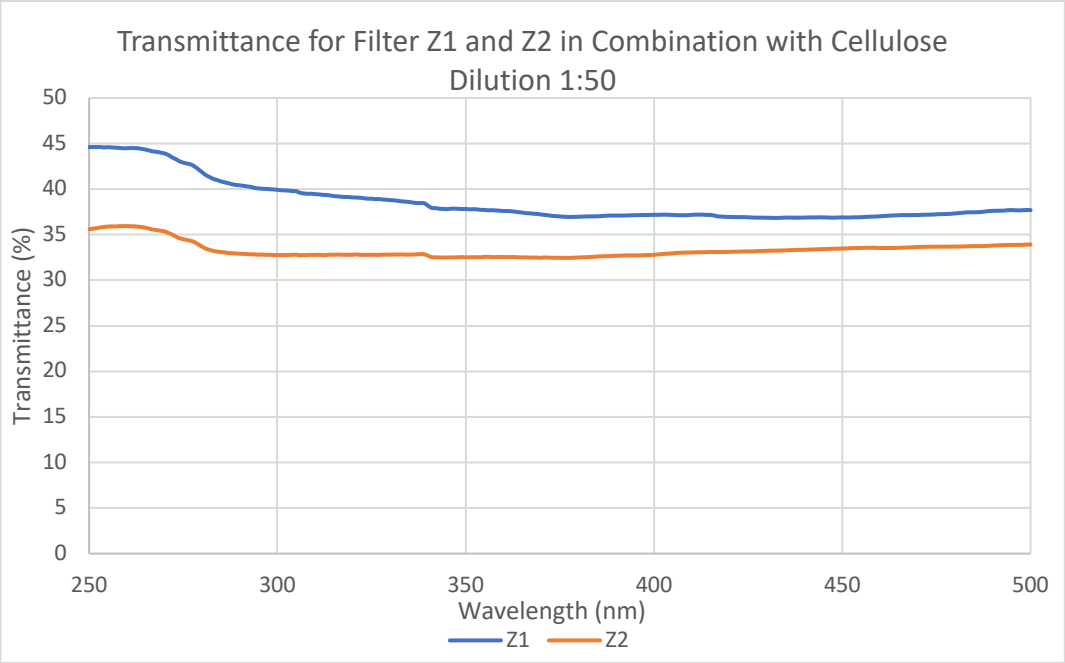


**Figure 17:** Transmittance spectrum for Filter Z1 and Z2 in combination with cellulose, diluted 1:50 in GDB with a blank containing the base moisturizer diluted 1:50 in GDB.

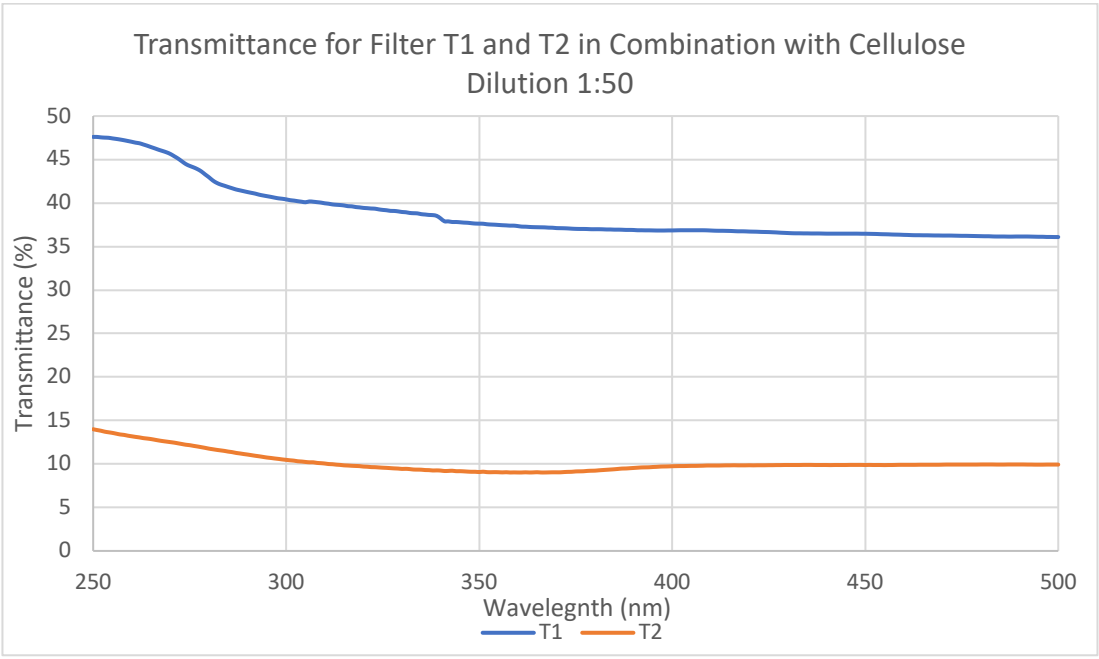


**Figure 18:** Transmittance spectrum for Filter T1 and T2 in combination with cellulose, diluted 1:50 in GDB with a blank containing the base moisturizer diluted 1:50 in GDB.

## C. Evaluation Form

Link and images of the evaluation form provided to the volunteering participants:

https://forms.gle/HGV3ppHvyPrHCjm17

# Evaluation of three Moisturizing SPF's

You are welcome to participate in an evaluation of three different moisturizing SPF's for us to proceed in the development of an enzyme-based moisturizing SPF for the face containing UV filters for sun protection. This is an anonymous onetime evaluation where you get to test three different product samples (all with an SPF of approximately 15) and answer the questions below concerning both theoretical and practical preferences. This development is also part of a Master's Thesis at Lund University and answers and results will be presented anonymously in the report.

Traditional moisturizers containing SPF may have a few problems, including:

- Not provide adequate sun protection, especially if the SPF is low or not applied correctly. This can lead to sunburn, premature aging, and an increased risk of skin cancer.
- Can feel heavy and greasy on the skin, which can be uncomfortable, especially in warm weather.
- Can leave a white cast on the skin, which may not be desirable for people with darker skin tones.
- May contain harsh chemicals or fragrances that can irritate sensitive skin, leading to rashes, redness, or acne breakouts.
- May not be compatible with other skincare products, such as serums or facial oils, which can lead to pilling or flaking.
- May not provide enough hydration for dry or dehydrated skin, leading to flakiness and dullness.

We have addressed these issues and developed an enzyme-based moisturizing SPF, a lightweight, non-greasy formula that provides broad-spectrum protection and should be compatible with your other skincare products. Our moisturizing SPF contains smart enzymes that work to gently exfoliate and renew your skin, promoting a healthy, glowing complexion and herbal extract which has a clinically proven long lasting hydration effect. The SPF provides broad-spectrum protection against harmful UVA and UVB rays, helping to prevent sunburn and premature aging.

**Ingredients:**

Following ingredients are included in all three samples: Glycerin, Aqua, Xylitol, Pentylene Glycol, Carbomer, *Imperata Cylindrica* Root extract, Protease, Tromethamine, PEG8, and Calcium Chloride.

In addition to these ingredients, the three samples contain different UV filters with following ingredients (the order below is not connected to the sample order in any way):

1. Titanium Dioxide, Aqua, Polyglyceryl-2 Caprate, Sucrose Stearate, Simmondsia Chinensis (Jojoba) Seed Oil, Stearic Acid, Alumina, Glyceryl Caprylate and Squalane
2. Zinc Oxide, C12-15 Alkyl Benzoate and Polyhydroxystearic Acid
3. C12-15 Alkyl Benzoate, Titanium Dioxide, Aluminium Stearate, Polyhydroxystearic Acid and Alumina

**Directions:**
The evaluation is divided into two sections, the first section is theoretical questions while the second section includes testing of the three different samples. Please read trough each section before testing the products to gain a better understanding of what aspects and parameters are to be evaluated.

- Test the products on the back of your hands and let the product sit for at least 10 minutes before eventually washing it off and testing a new sample.
- Do not apply too much when testing, one or two drops (maximum half a pump stroke) is enough for the back of the hand.
- Make sure to work in the product thoroughly for best sensation and appearance.
- Do not apply one sample over another, if you want to test the next sample on the same place as the previous one, please wash off the first one.

I hereby agree to take part in this evaluation and that my answers can be used for further development of this moisturizing SPF and be presented anonymously in a Master's Thesis Report *

If there are any adverse reactions, we advise for you to let us know and halt the evaluation entirely. You can withdraw any time for any reason but please inform us.

Contact persons: Karolina Torfgård (karolina.torfgard@telia.com) or Dietlind Adlercreutz (dietlind.adlercreutz@zymiq.com)

- ○ Yes

Gender *

- ○ Man
- ○ Woman
- ○ Other
- ○ Do not want to say

Age *

- ○ ≤20
- ○ 21-40
- ○ 41-60
- ○ >60

Which country and city do you live in?

Short answer text

How often do you use a moisturizing face SPF? *

- ○ Twice a day (or more)
- ○ Once a day
- ○ A couple of times a week
- ○ A couple of times a month
- ○ Never

How important would you consider the following parameters when buying a moisturizing face SPF? Please rank from 1 (Least important) to 6 (Most important). *

| | 1 | 2 | 3 | 4 | 5 | 6 |
|---|---|---|---|---|---|---|
| Protects aga... | ○ | ○ | ○ | ○ | ○ | ○ |
| Environment... | ○ | ○ | ○ | ○ | ○ | ○ |
| Does not giv... | ○ | ○ | ○ | ○ | ○ | ○ |
| Smooth feeli... | ○ | ○ | ○ | ○ | ○ | ○ |
| No sticky fe... | ○ | ○ | ○ | ○ | ○ | ○ |
| Low price | ○ | ○ | ○ | ○ | ○ | ○ |

What number of SPF would you prefer in a daily moisturizing face SPF? *

- ○ SPF 15
- ○ SPF 30
- ○ SPF 40+

To be able to compare the results from the evaluation, parameters are clarified below to increase the understanding of what is asked for.

- **Wetness:** How wet does the sample both feel and look when applied. (1 = Not wet att all, 5 = Watery feeling and appearance)
- **Spreadability:** How well/easily does the sample spread out when applied (1 = Very hard to spread out, 5 = Super easy to spread out)
- **Smoothness:** How smooth does the sample feel on the skin when applied (1 = Not smooth at all, 5 = Super smooth)
- **Thickness:** How thick is the consistency of the sample when pumped out and applied (1 = Very runny, 5 = Very thick)
- **White cast:** How much of a white cast (white residues on the skin) does the sample leave on your skin, when first applied/spread out and after 10 minutes. (1 = No white cast at all, 5 = Thick white layer)
- **Stickiness:** How sticky does the sample feel when first applied/spread out and after 10 minutes (1 = Completely dry, 5 = Super sticky)
- **Heavy- and greasiness:** How heavy and greasy on the skin does the sample feel compared to each other and to other sunscreens and moisturizers used previously. (1 = Light weight moisturizer, 5 = Conventional beach sunscreen)

Directions repeated from previous section:

- Test the products on the back of your hands and let the product sit for at least 10 minutes before eventually washing it off and testing a new sample.
- Do not apply too much when testing, one or two drops (maximum half a pump stroke) is enough for the back of the hand.
- Make sure to work in the product thoroughly for best sensation and appearance.
- Do not apply one sample over another, if you want to test the next sample on the same place as the previous one, please wash off the first one.

Please evaluate Sample A considering the parameters stated below. Rank them from 1 to 5 where 1 is the lowest and 5 is the highest. *

| | 1 | 2 | 3 | 4 | 5 |
|---|---|---|---|---|---|
| Wetness | ○ | ○ | ○ | ○ | ○ |
| Spreadability | ○ | ○ | ○ | ○ | ○ |
| Smoothness | ○ | ○ | ○ | ○ | ○ |
| Thickness | ○ | ○ | ○ | ○ | ○ |
| White cast | ○ | ○ | ○ | ○ | ○ |
| White cast afte... | ○ | ○ | ○ | ○ | ○ |
| Stickiness | ○ | ○ | ○ | ○ | ○ |
| Stickiness afte... | ○ | ○ | ○ | ○ | ○ |
| Heavy- and gre... | ○ | ○ | ○ | ○ | ○ |

Please evaluate Sample B considering the parameters stated below. Rank them from 1 to 5 where 1 is the lowest and 5 is the highest. *

| | 1 | 2 | 3 | 4 | 5 |
|---|---|---|---|---|---|
| Wetness | ○ | ○ | ○ | ○ | ○ |
| Spreadability | ○ | ○ | ○ | ○ | ○ |
| Smoothness | ○ | ○ | ○ | ○ | ○ |
| Thickness | ○ | ○ | ○ | ○ | ○ |
| White cast | ○ | ○ | ○ | ○ | ○ |
| White cast afte... | ○ | ○ | ○ | ○ | ○ |
| Stickiness | ○ | ○ | ○ | ○ | ○ |
| Stickiness afte... | ○ | ○ | ○ | ○ | ○ |
| Heavy- and gre... | ○ | ○ | ○ | ○ | ○ |

Please evaluate Sample C considering the parameters stated below. Rank them from 1 to 5 where 1 is the lowest and 5 is the highest. *

| | 1 | 2 | 3 | 4 | 5 |
|---|---|---|---|---|---|
| Wetness | ○ | ○ | ○ | ○ | ○ |
| Spreadability | ○ | ○ | ○ | ○ | ○ |
| Smoothness | ○ | ○ | ○ | ○ | ○ |
| Thickness | ○ | ○ | ○ | ○ | ○ |
| White cast | ○ | ○ | ○ | ○ | ○ |
| White cast afte... | ○ | ○ | ○ | ○ | ○ |
| Stickiness | ○ | ○ | ○ | ○ | ○ |
| Stickiness afte... | ○ | ○ | ○ | ○ | ○ |
| Heavy- and Gre... | ○ | ○ | ○ | ○ | ○ |

Which of the samples tested would you most likely buy considering all aspects evaluated? *
Please rank from 1 (Least likely) to 4 (Most likely).

| | 1 | 2 | 3 | 4 |
|---|---|---|---|---|
| Sample A | ○ | ○ | ○ | ○ |
| Sample B | ○ | ○ | ○ | ○ |
| Sample C | ○ | ○ | ○ | ○ |
| None of them | ○ | ○ | ○ | ○ |

Do you have anything to add considering your experience with the testing of these samples?

Long answer text

Double click on the image below to open the results from the evaluation form.

| I hereby agre | Gender | Age | Which country a | How often do y | How important | How important | How important | How important | How important | How important | What number o | Please evaluate | Please evaluate | Please evaluate | Please evaluate | P |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Yes | Woman | 21-40 | Sweden | Once a day | 6 | 2 | 3 | 1 | 5 | 4 | SPF 30 | 4 | 5 | 4 | 1 | |
| Yes | Woman | 41-60 | Sweden, Kullavi | Once a day | 4 | 3 | 5 | 2 | 6 | 1 | SPF 15 | 2 | 5 | 4 | 2 | |
| Yes | Man | 21-40 | Sweden | Once a day | 6 | 3 | 2 | 1 | 5 | 4 | SPF 30 | 4 | 4 | 3 | 2 | |
| Yes | Woman | 21-40 | London | A couple of time | 6 | 1 | 5 | 4 | 3 | 2 | SPF 40+ | 4 | 5 | 3 | 1 | |
| Yes | Woman | 21-40 | London, UK | Once a day | 6 | 2 | 5 | 3 | 4 | 1 | SPF 40+ | 3 | 5 | 5 | 3 | |
| Yes | Man | 21-40 | France | Never | 6 | 5 | 4 | 2 | 3 | 1 | SPF 15 | 4 | 5 | 4 | 4 | |
| Yes | Man | 41-60 | Sweden | Never | 6 | 3 | 1 | 4 | 5 | 2 | SPF 30 | 4 | 5 | 4 | 2 | |
| Yes | Man | 21-40 | Sweden, Lund | A couple of time | 6 | 4 | 3 | 2 | 5 | 1 | SPF 40+ | 5 | 5 | 4 | 1 | |
| Yes | Woman | 21-40 | London | Once a day | 6 | 1 | 5 | 2 | 3 | 4 | SPF 40+ | 2 | 5 | 5 | 2 | |
| Yes | Woman | ≤20 | Sweden, Landsk | Once a day | 6 | 5 | 4 | 1 | 2 | 3 | SPF 30 | 2 | 5 | 5 | 4 | |
| Yes | Woman | 21-40 | London UK | Twice a day (or | 6 | 3 | 5 | 4 | 1 | 2 | SPF 40+ | 4 | 3 | 4 | 4 | |
| Yes | Woman | 21-40 | Sweden | Never | 6 | 4 | 5 | 2 | 3 | 1 | SPF 30 | 3 | 4 | 4 | 3 | |
| Yes | Man | 21-40 | United Kingdom | A couple of time | 6 | 1 | 2 | 4 | 5 | 3 | SPF 30 | 3 | 5 | 4 | 3 | |
| Yes | Man | 21-40 | London | Never | 2 | 1 | 6 | 5 | 4 | 3 | SPF 30 | 5 | 5 | 5 | 5 | |
| Yes | Woman | 21-40 | Sweden, Lund | Once a day | 4 | 2 | 6 | 1 | 5 | 3 | SPF 30 | 3 | 4 | 4 | 1 | |
| Yes | Woman | 21-40 | Sweden, Lund | Once a day | 2 | 4 | 1 | 3 | 6 | 5 | SPF 15 | 4 | 5 | 4 | 3 | |
| Yes | Woman | 21-40 | Lund | Once a day | 6 | 1 | 5 | 3 | 4 | 2 | SPF 40+ | 4 | 4 | 3 | 1 | |
| Yes | Woman | 21-40 | Sweden | Once a day | 5 | 2 | 4 | 3 | 6 | 1 | SPF 30 | 4 | 3 | 3 | 3 | |
| Yes | Woman | 21-40 | Sweden and Lu | Once a day | 3 | 2 | 5 | 1 | 6 | 4 | SPF 30 | 4 | 2 | 1 | 1 | |
| Yes | Woman | 21-40 | Sweden | A couple of time | 6 | 5 | 2 | 3 | 1 | 4 | SPF 30 | 4 | 5 | 3 | 2 | |
| Yes | Man | 21-40 | London | A couple of time | 6 | 2 | 4 | 5 | 3 | 1 | SPF 40+ | 3 | 4 | 5 | 3 | |
| Yes | Man | >60 | Sweden, Lund | Never | 6 | 2 | 3 | 4 | 5 | 1 | SPF 30 | 3 | 4 | 5 | 3 | |
| Yes | Man | 21-40 | Denmark | Once a day | 3 | 2 | 3 | 3 | 3 | 1 | SPF 15 | 4 | 5 | 4 | 2 | |
| Yes | Woman | 21-40 | denmark | Once a day | 4 | 5 | 6 | 4 | 6 | 5 | SPF 15 | 4 | 5 | 4 | 2 | |
| Yes | Woman | 21-40 | Copenhagen, D | A couple of time | 6 | 5 | 4 | 2 | 5 | 2 | SPF 30 | 3 | 4 | 5 | 4 | |
| Yes | Woman | 21-40 | Copenhagen | Once a day | 3 | 1 | 3 | 2 | 3 | 2 | SPF 40+ | 3 | 5 | 5 | 3 | |
| | | | | | 5.076923077 | 2.730769231 | 3.884615385 | 2.730769231 | 4.115384615 | 2.423076923 | | 3.538461538 | 4.461538462 | 4 | 2.5 | |