



M 2023

INCORPORATION OF ONION PEEL EXTRACTS AS AN ALTERNATIVE TO SYNTHETIC UV FILTERS TO DEVELOP A VALUE-ADDED SUNSCREEN

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PRESENTED TO THE FACULTY OF ENGINEERING
OF THE UNIVERSITY OF PORTO

Master in Chemical Engineering

Incorporation of Onion Peel Extracts as an alternative to synthetic UV filters to develop a value-added sunscreen

Master dissertation

of

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Developed within the course of dissertation

held in

LEPABE/ALiCE/ FEUP



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July 2023

Acknowledgment

Firstly, I would like to express my gratitude towards my supervisor, Prof. Dr. Lúcia Santos, for the continuous advice, incredible support and infinite patience during the last few months. My appreciation also extends to Dr. Loleny Tavares, for the incredible support and advice throughout these months.

I couldn't be more thankful for the people in E201, you are incredible and made these months more enjoyable and less stressful. I would like to grant my special gratitude towards Eng. Sara Ferreira and Eng. Sandra Gomes. Sara, without your guidance, mentoring, support and patience, this dissertation would not have happened. Sandra, I will always remember your support and care over these last few months. I am forever grateful for ever encountering the both of you and having the pleasure of working beside you.

I would like to extend my gratitude towards laboratory technicians for their constant help and availability, in particular to Fátima, Eng. Zé Luís and Dr. Luís Carlos.

My gratitude towards João Manuel Calado, who kindly provided the onion peels used in this dissertation.

I would like to dedicate a special thanks to my friends for your incredible patient, for listening to me and offering me all the support and care in the world. A special thank you to Inês Brito.

To my family, who always encouraged me to follow my dreams and never give up.

I also place on record my sense of gratitude to all those who contributed directly or indirectly to the dissertation.

This work was financially supported by: LA/P/0045/2020 (ALiCE), UIDB/00511/2020 and UIDP/00511/2020 (LEPABE), funded by national funds through FCT/MCTES (PIDDAC);

Project S4Hort_Soil&Food – Sustainable practices for Soil health & horticultural products quality improvement in the Entre Douro e Minho Region, with reference NORTE-01-0145-FEDER-000074, co-financed by the European Regional Development Fund (ERDF), through the North Portugal Regional Operational Programme (NORTE2020), under the PORTUGAL 2020 Partnership Agreement.

Abstract

The escalating concern about the detrimental ramifications of synthetic UV filters in sunscreens stems from the potential harm and toxicity of these compounds to both aquatic organisms and humans. Consequently, there is some urgency in identifying novel compounds that can serve as UV filters while ensuring safety and reduced environmental impact. In this regard, plant extracts containing phenolic compounds have emerged as a promising and sustainable alternative. This is attributed to their significant antioxidant capacity and UV filters. The scope of this project was the characterization of the antioxidant and photoprotection properties of the onion peel (OP) extracts, both non encapsulated and microencapsulated, and the production and evaluation of a sunscreen formulation incorporating both extracts.

The characterization of the extract revealed a great antioxidant capacity that was evaluated by calculating the total phenolic content of the extract, $(585.23 \pm 10.43) \text{ mg}_{\text{GAE}} \text{ g}^{-1}_{\text{extract}}$, by 2,2-diphenyl-1-picrylhydrazyl (DPPH) test, $(199.71 \pm 1.16) \text{ mg}_{\text{TE}} \text{ g}^{-1}$, and 2,2'-Azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) assay, $(381.45 \pm 8.05) \text{ mg}_{\text{TEAC}} \text{ g}^{-1}$. The results fall within the range of values found in the literature. High-performance liquid chromatography (HPLC) was employed to identify and quantify the main phenolic compounds of the OP extract. The results revealed a significant presence of quercetin and resveratrol. The photoprotection capacity was evaluated by calculating the sun protection factor (SPF). SPF studies revealed that solutions containing OP extract provide significantly less photoprotection than solutions containing the synthetic UV filter, for the same mass concentration. However, the phenolic extract is a mixture of compounds as opposed to a single compound as the oxybenzone, suggesting a need for higher concentrations. The results demonstrated that increasing the concentration of OP extract solutions led to a higher value of SPF than that obtained for the synthetic UV filter, implying that OP extract has high potential as a natural UV filter. OP extract was microencapsulated by molecular inclusion, using β -cyclodextrin as coating agent. The microencapsulated process revealed an encapsulation efficiency of $(91.80 \pm 0.03)\%$, a mean diameter of $(30.84 \pm 22.14) \mu\text{m}$, and a size distribution with a polydispersity of 2.09. Microparticles appeared in irregular shape and have a porous surface, which might reveal a potential release method. When compared to the non-encapsulated extract, microencapsulated demonstrated a higher antioxidant capacity, but lower photoprotection capacity.

Additionally, both extracts were incorporated into oil-in-water emulsions. The pH of the formulations revealed values within the normal range for topical application, and all the formulations were stable along time. The formulations containing OP extract demonstrated great antioxidant capacity in opposition to the negative and positive control (containing synthetic UV filter) that showed no antioxidant capacity after 5 weeks. Regarding their photoprotection properties, the results showed that OP extract (non-encapsulated and encapsulated) possesses photo protective capacities. Microencapsulated extract displayed a higher ability to maintain the SPF value along 5 weeks, potentially increasing the shelf life of the formulation.

In conclusion, OP extract (non-encapsulated and microencapsulated) displays the potential to be used as natural UV filter to produce a value-added sunscreen.

Keywords (theme): sunscreen, onion peel, phenolic compounds, UV filters, microencapsulatio

Resumo

As crescentes evidências de que os filtros solares sintéticos podem ser nocivos e tóxicos para os seres humanos e para o meio aquático, têm levado a um crescente esforço da comunidade científica para encontrar alternativas sustentáveis. Novos compostos naturais, seguros, não poluentes e com capacidades foto protetoras aparecem como alternativa aos filtros solares sintéticos. Os extratos de subprodutos agrícolas, ricos em compostos fenólicos, têm-se revelado uma alternativa promissora e sustentável aos filtros UV sintéticos, devido às suas propriedades fotoprotetoras e também elevada capacidade antioxidante. No entanto, estes compostos são muito sensíveis à luz e ao pH, pelo que a microencapsulação surge como solução para evitar a sua degradação quando incorporados em formulações cosméticas. Este projeto tem como objetivo caracterizar as propriedades antioxidantes e fotoprotetoras dos extratos de casca de cebola (OP), livre e microencapsulado, bem como a produção e avaliação de formulações de proteção solar, contendo ambos os extratos.

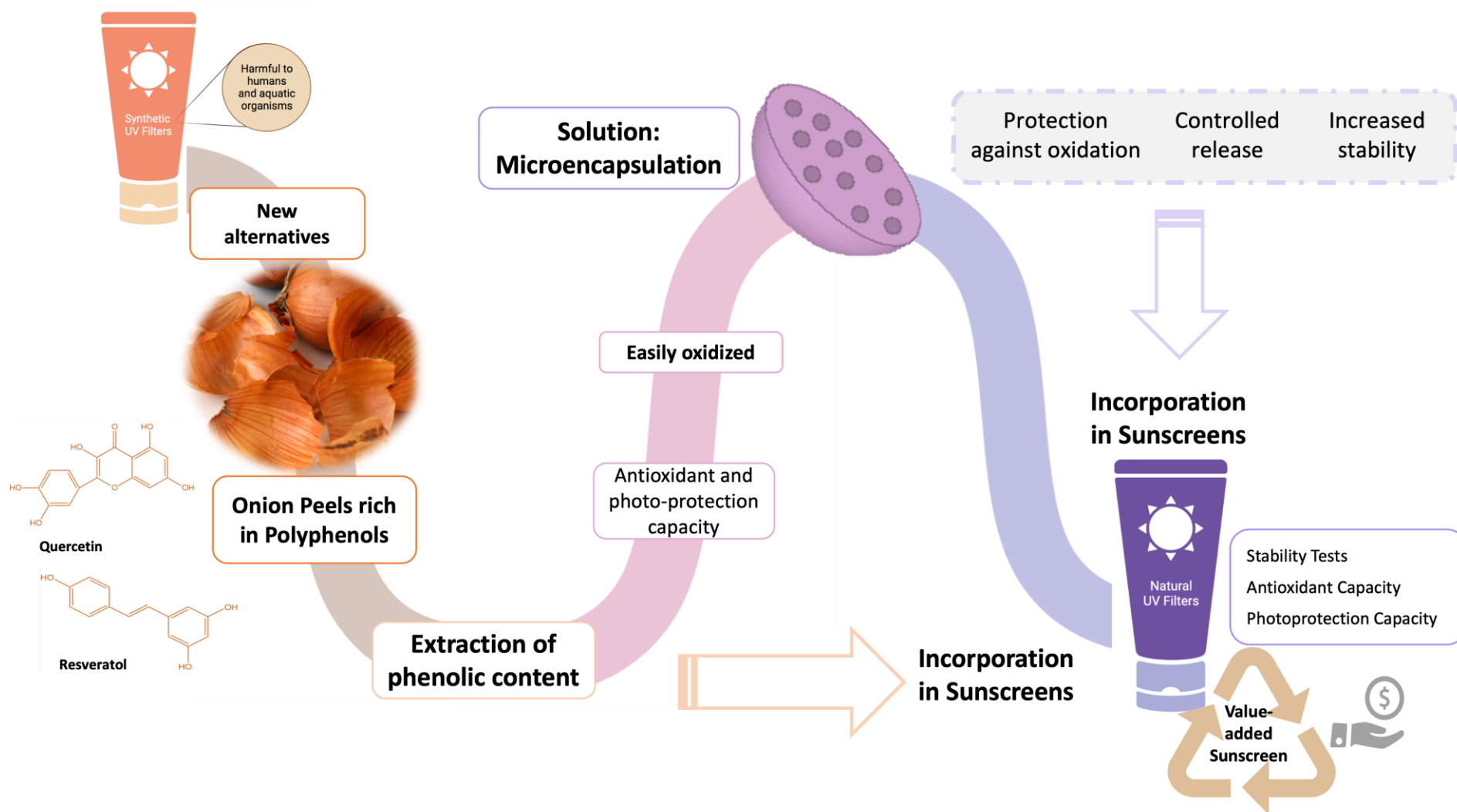
A caracterização do extrato de OP demonstrou alta capacidade antioxidante, que foi avaliada pelo cálculo do conteúdo fenólico total do extrato, $(585,23 \pm 10,43) \text{ mg}_{\text{GAE}} \text{ g}^{-1}_{\text{extract}}$, e pelos ensaios de 1,1-difenil-2-picril-hidrazil (DPPH), $(199,71 \pm 1,16) \text{ mg}_{\text{TE}} \text{ g}^{-1}$, e 2,2-azinobis (3-etilbenzotiazolina-acidosulfónico) (ABTS), $(381,45 \pm 8,05) \text{ mg}_{\text{TEAC}} \text{ g}^{-1}$. Por cromatografia líquida de alta performance quantificaram-se os principais compostos fenólicos do extrato de OP. Os resultados revelaram uma presença significativa de quercetina e resveratrol. A capacidade fotoprotetora foi averiguada através do cálculo do fator de proteção solar (SPF). Os estudos sobre o SPF revelaram que para soluções da mesma concentração, o extrato proporciona uma fotoproteção significativamente menor do que as soluções que contêm o filtro UV sintético. Contudo, o extrato fenólico é uma mistura de compostos, ao contrário da oxibenzona (filtro sintético), o que sugere a necessidade de se utilizar em concentrações mais elevadas. Os resultados demonstraram que, ao aumentar a concentração das soluções de extrato de OP, obteve-se um valor de SPF superior ao obtido para o filtro sintético, o que permite inferir que o extrato de OP tem um elevado potencial como filtro solar natural. O extrato de OP foi microencapsulado, por inclusão molecular, utilizando β -ciclodextrina como agente encapsulante. O processo de microencapsulação revelou uma eficiência de encapsulação de $(91,80 \pm 0,03)\%$, um diâmetro médio de $(30,84 \pm 22,14) \mu\text{m}$ e uma distribuição de tamanho com uma polidispersão de 2,09. As micropartículas apresentaram uma forma irregular e uma superfície porosa, o que pode revelar um potencial método de libertação dos compostos presentes no extrato. Quando comparado com o extrato livre, o extrato microencapsulado demonstrou uma maior capacidade antioxidante, mas uma menor capacidade fotoprotetora.

Ambos os extratos foram incorporados em emulsões de óleo em água. O pH das formulações revelou valores dentro da gama normal para aplicação tópica e todas as formulações foram consideradas estáveis. As formulações contendo extrato de OP demonstraram uma grande capacidade antioxidante, em oposição ao controlo negativo e positivo (contendo filtro UV sintético). Os resultados permitem concluir que o extrato de OP, quer livre ou microencapsulado, oferece fotoproteção. O extrato microencapsulado manteve um valor de SPF mais elevado durante 5 semanas, aumentando potencialmente o tempo de prateleira da formulação.

Em conclusão, o extrato de OP (livre e microencapsulado) possui potencial para ser utilizado como filtro solar natural na produção de um protetor solar de valor acrescentado.

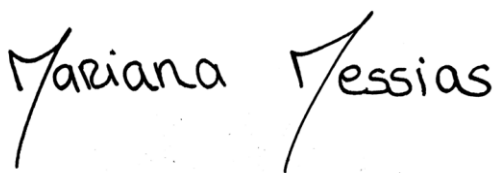
Palavras chave: protetor solar, cascas de cebolas, compostos fenólicos, filtros solares, microencapsulação

Graphical Abstract



Declaration

I, Mariana Aranha Messias, hereby declare, under word of honor, that this work is original and that all non-original contributions are indicated and due reference is given to the author and source.

A handwritten signature in black ink that reads "Mariana Messias". The signature is written in a cursive style with a large, stylized 'M' and 'M'.

Oporto, 3rd July 2023

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Notation and Glossary

A	Peak Area	
Abs	Absorbance	
C	Concentration	kg m^{-3}
$Dv, 10$	Maximum particle diameter below which 10% of the sample volume exists	m
$Dv, 50$	Maximum particle diameter below which 50% of the sample volume exists	m
$Dv, 90$	Maximum particle diameter below which 90% of the sample volume exists	m
m	Mass	kg
P	Pressure	Pa
T	Temperature	K
t_0	First time of analysis of sunscreens	s
t_1	Second time of analysis of sunscreens	s
t_2	Third time of analysis of sunscreens	s

Greek Letters

σ	standard deviation	
λ	wavelength	m

Indexes

0	Calculated
a	Detected
b	Initially used
blank	Blank
control	Control
f	Used
GA	Gallic acid
GAE	Gallic Acid Equivalents
OP	Onion Peels
sample	Sample

List of Acronyms

ABTS	2,2'-Azino-bis(3-ethylbenzothiazoline-6-sulfonic acid)
BHT	Butylated Hydroxytoluene
CD	Cyclodextrin
CF	Correction factor
DAD	Diode array
DSC	Differential Scanning Calorimetry
DPPH	2,2-diphenyl-1-picrylhydrazyl
EE	Encapsulation Efficiency
FEUP	Faculty of Engineering of University of Porto
FDA	Food and Drug Administration
GAE	Gallic Acid Equivalents
IC ₅₀	half maximal inhibitory concentration
HAT	Hydrogen Atom Transfer
HPLC	High Performance Liquid Chromatography
LOD	Limit of Detection
LOQ	Limit of Quantification
MP-OP	Microencapsulated Onion Peel extract
MP-OPE	Microencapsulated Onion Peel Extract Formulation
MIX	Mixture Formulation
NC	Negative Control
OP	Onion Peel

OPE	Onion Peel Extract Formulation
O/W	Oil-in-water
PC	Positive control
PDI	Polydispersity Index
PVDF	Polyvinylidene difluoride
PY	Product Yield
RSC	Radical Scavenging Capacity
SEM	Scanning Electron Microscopy
SET	Single Electron Transfer
SPF	Sun Protection Factor
TE	Trolox equivalents
TEAC	Trolox equivalents antioxidant capacity
TGA	Thermogravimetric Analysis
TPC	Total Phenolic Contents
UV	Ultraviolet
UVR	Ultraviolet Radiation
W/O	Water-in-oil

1 Introduction

In recent years, frequent extreme climate occurrences, in conjugation with infectious diseases outbreaks (such as COVID-19 and influenza A) has led to an increase in the global health awareness and shifts in consumers' habits to minimize human impact on the planet and to improve health (Baker et al., 2022; Venghaus et al., 2022).

Skin is the first barrier between human body and external environment and its harms, such as radiation. Furthermore, it also presents a significant role in a person's physical appearance which is the reason behind the interest in keeping it healthy and glowing (Hughes et al., 2022; A. S. Ribeiro et al., 2015).

Sunlight exposure is essential for the human body to produce vitamin D and induction of β -endorphin expression and so people are advised to spend at least 20 min a day outside. However, unprotected and prolonged sun exposure is harmful for the skin and can cause several dermatological conditions such as solar sunburn, hyperpigmentation, and skin cancer (Jesus et al., 2022; Lindqvist et al., 2022). Furthermore, ultraviolet (UV) radiation also produces free radicals in the skin. Free radicals are molecules that damage the skin cells and can cause further conditions and diseases, and so it is also very important for sunscreens to have antioxidants to neutralize them (Lorigo & Cairrao, 2019).

In order to have the benefits and minimize the harm of the sunlight exposure people are using sunscreens more often, which makes sunscreens one of the most sought and consumed skincare products in the world.

There are three types of solar UV radiation in solar radiation: C, B and A, as illustrated in Figure 1.1.

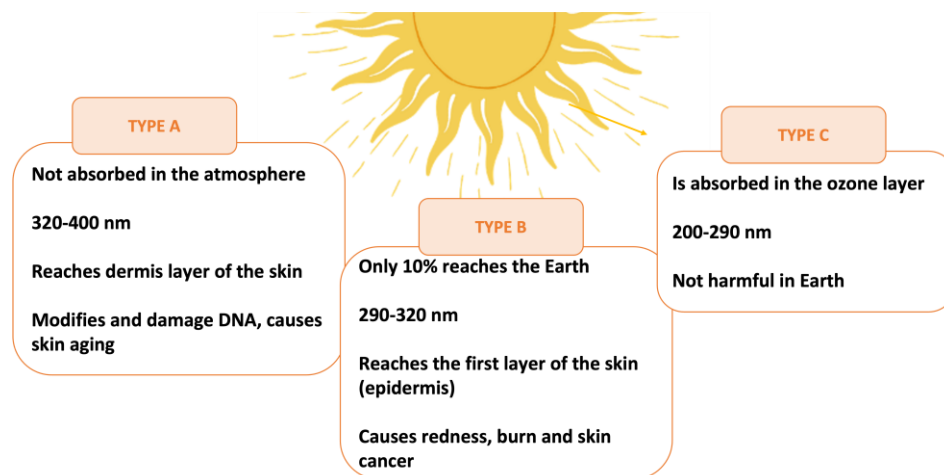


Figure 1.1 - Types of UV radiation and its effects on skin.

Sunscreens are efficient if they contain UV filters to combat both type B and type A UV radiation. There are several synthetic UV filters in the market, which have been studied and approved by different regulatory associations. However, concerns about its safety towards the environment have been raised in recent years, such as its noxious effect towards marine species, leading to some UV filters being removed from the market (Jesus et al., 2022).

As an alternative to synthetic UV filters, natural compounds rich in antioxidants and with photoprotector capacity, have been gaining interest from scientists, such as plant extracts rich in polyphenols, flavonoids and phenolic acids. For example, resveratrol (polyphenol) and quercetin (flavonoid) are known for their high capacity to absorb UV radiation, and so, are possible to be used as natural UV filters (Ferreira et al., 2023).

A source of plant extracts bio-compounds to be considered is agricultural by-products. As the population of the world grows exponentially, so does the waste produced by it. This waste is usually poorly managed, being an enormous pollutant of the planet. Thereby, finding a way to treat, reutilize or properly dispose of this waste is of utter importance (Koul et al., 2022). Furthermore, these by-products are cheap and easy to obtain, since they are discarded by restaurants and industries. In addition, some by-products are phytotoxic and biotoxic due to their high content of phenolic compounds (Paulo & Santos, 2021). In this sense, by adding another step in their life time and incorporating their extracts in cosmetic formulations, the bioactive compounds present in the extract are valued, minimizing their harm to the environment. One example of an agricultural by-product is onion peel, which is rich in bio-compounds, such as quercetin and resveratrol (Ferreira et al., 2023).

The polyphenolic compounds in most of these by-products, though having great antioxidant properties and photo protectant capacity, are highly sensitive to light, pH, temperature and/or oxygen, being oxidized and losing their bioactive interest. Therefore, there is a need to protect them and keep them active longer in the formulations. A suggested approach is microencapsulation. This method guarantees an increased stability of the phenolic compounds, controlled release of the active compounds, protection against degradation and hides/minimizes the effect of possible undesirable sensory properties of these agents (Böger et al., 2022).

Therefore, the possibility of creating a value-added facial moisturizer with SPF, using organic natural UV filters, should be explored and studied.

The present study aims to:

- Obtain the extract from onion peels and its characterization regarding antioxidant properties as well as sun protection capacity;
- Microencapsulate the extract and characterize the resulting microparticles;
- Incorporate both the non-encapsulated and microencapsulated onion peel extract in a sunscreen formulations and analyze their properties;
- Characterize both formulations and compare their antioxidant properties, sun protection capacity and stability with the formulation using microparticles.

1.1 Contribution of the Author for the work

All the experimental work was performed by the author, except: validation of the Gallic acid calibration curve and validation of the HPLC method.

Validation of the Gallic acid calibration curve and validation of the HPLC method were performed by Eng. Sara Ferreira and Eng. Sandra Gomes.

1.2 Organization of the dissertation

This dissertation has 6 chapters, to which are added Annexes, Appendixes and References. Introduction, the first chapter, presents the issue of the project includes a study overview. The second chapter, Context and State of Art, introduces the subject focusing on sunscreens and UV filters, addressing the topic of by-products, their properties as antioxidants and UV filters and the legislation concerning their use as UV filters in cosmetics. Additionally, it is also explored the use of microparticles as a protection of the extracts against degradation. The third chapter, Materials and Methods, provides information on the materials and equipment used during the work, as well as the protocols followed. Results and Discussion, the fourth chapter, discusses the obtained results. The fifth chapter, Conclusion, presents the conclusions drawn from the project. The sixth chapter, Assessment of the work done, discusses the dissertation's limits and presents suggestions as what should be done as further work. Annexes and Appendixes contain additional information that may be necessary to understand the full extent of the work developed. References present the literature used and followed in this dissertation.

2 Context and State of the art

2.1 Sunscreens

Although the first sunscreen “*Amber Solaire*” was only commercialized in 1935, ancient communities have been using natural photoprotectors, usually plant extracts, for a long time to prevent sunburns. With time, new synthetic formulations emerged and sunscreens as we know them today were developed (Jesus et al., 2022).

While in Europe sunscreens are considered a cosmetic product, in the United States of America, they are regulated as over-the-counter drugs (medicinal products) as they prevent disease or skin injury (Parliament, 2009). Sunscreen is a cosmetic product that protects the skin from UV radiation using UV filters (Ferreira et al., 2023).

When developing a sunscreen, there are several requirements that have to be met, such as inertness, non-irritant, photostability, and compatibility with other ingredients/cosmetic products. Sunscreens formulations should also have low viscosity, be aesthetically appealing and present good spreadability. Finally, they must provide protection against UVA and UVB radiation as well as not being highly absorbed to prevent skin sensibility (Geoffrey et al., 2019). There are several types of sunscreen formulations and are required by the consumer depending on skin type, age and filter type, and are presented in Figure 2.1.



Figure 2.1 - Different types of sunscreen formulations.

The most common sunscreens formulations are emulsions, either oil-in-water (O/W) or water-in-oil (W/O). O/W emulsions consist of oil droplets in an aqueous phase and W/O consist of water droplets in an oil phase (Acker et al., 2014). Oil-in-water formulations are usually not heavy on skin and are less occlusive than water-in-oil formulations, which makes them the consumer preference (Ferreira et al., 2023). Furthermore, sunscreen formulations contain several other ingredients as depicted in Figure 2.2, in order to perform all the functions required. such as being non-toxic, antioxidant, moisturizing and protecting against UVA and UVB radiation (Ferreira et al., 2023).

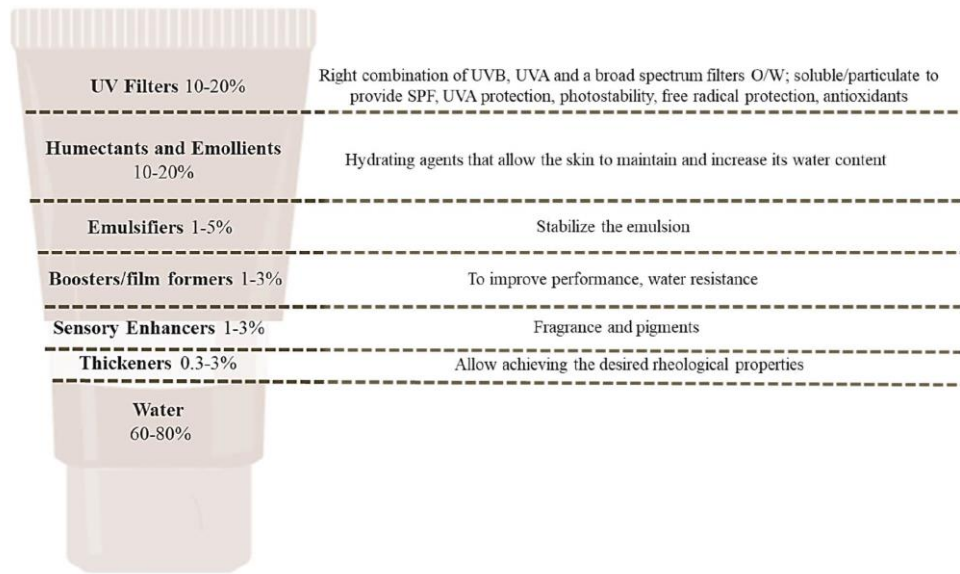


Figure 2.2 - Sunscreen formulations main ingredients from (Ferreira et al., 2023) .

As shown in Figure 2.2, one of the main ingredients of sunscreens are UV filters.

2.1.1 UV filters

UV filters are the barrier that protects human skin from UV radiation, and can either absorb only UVA or UVB radiation or absorb both types of UVR and be classified as broad-spectrum. UV filters can also be divided according to their method of blocking the radiation, being organic UV filters if they absorb UVR and inorganic if they reflect and scatter it (Jesus et al., 2022). Some of the most commonly used UV filters are presented in Figure 2.3.

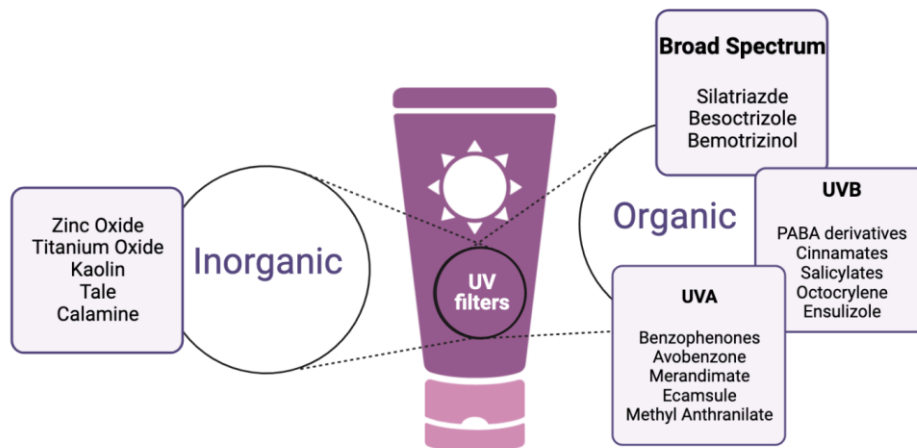


Figure 2.3 - Most widely used synthetic UV filters.

The last classification also applies to sunscreens: if they contain only organic filters they are classified as chemical sunscreen and if they only have inorganic filters are considered physical sunscreens. The differences of both types of sunscreens are presented in Table 2.1.

Table 2.1 - Differences between chemical and physical UV filters.

Chemical Sunscreen	Physical Sunscreen
Organic filter	Inorganic filter
Absorbs UV radiation converting it into heat	Reflects UV radiation acting like a barrier
Thin	Thick and leaves a white cast
Additives, like antioxidants, are easily added to the formulations	Less reactive and so it is the best option for sensitive skin
Active 20 to 30 min after applied	Immediately active

Chemical sunscreens are more widely accepted as they are more pleasant to apply and present better aesthetics. Nevertheless, they may be systemically absorbed and increase the sensitivity of the skin. Physical sunscreens, although not causing sensitivity, upon application may leave a white residue that is hard to spread, which leads to this type of sunscreen being rejected by the consumers based on aesthetic factors (Geoffrey et al., 2019). Nowadays, most sunscreens available in the markets are a mixture of both, containing organic and inorganic filters in order to enhance the compliance of consumers as well as balancing the protection against UV radiation (Geoffrey et al., 2019).

In recent years, concerns about the photostability, environmental impact and human toxicity of sunscreens (and UV filters) have arise (Jesus et al., 2022).

Photostability of sunscreen is mandatory in order to guarantee photoprotection and safety of the cosmetic. When subjected to UVR, UV filters absorb it and move to an excited energetic level. Then, the energy is released, and the molecules return to its base/original energetic level. During this process, some molecules suffer photoisomerization and cleavage of some bonds, leading to the degradation of the UV filters and, consequentially, sunscreens and to the loss of its properties. The derivatives of the modified molecules may also be toxic, damage the DNA or affect the stability of other constituents of the formulation (Ferreira et al., 2023; Jesus et al., 2022). Literature suggests different strategies to improve the photostability of UV filters, such as the use of antioxidants, microencapsulation of these and association of various UV filters (Jesus et al., 2022).

Concerns about the toxicity of sunscreens/UV filters to the human body and to the environment are increasing daily. Figure 2.4 presents the most affected systems in human body, as well as the most affected marine species.

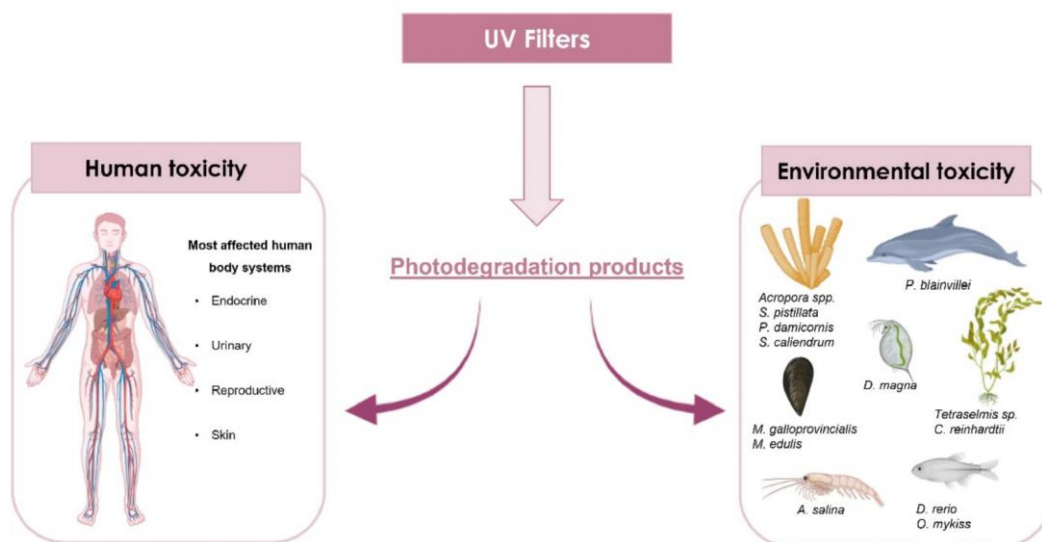


Figure 2.4- Main human systems and marine species harmfully affected by UV filters from (Jesus et al., 2022).

Even though, there are still few studies about human toxicity of sunscreens, it has been reported that UV filters are able to penetrate human skin and infiltrate in the blood, leading to concerns about its effect in other parts of the human body, namely its effect in endocrine and reproductive systems. A crucial factor in this is the size of the particles, especially nanoparticles whose size can induce cytotoxicity (Ferreira et al., 2023; Jesus et al., 2022).

The growth seen in use of sunscreen lead to a bioaccumulation of UV filters in marine environments that is affecting several marine species. Namely, bioaccumulation of UV filters in marine habitats has been proven to affect corals growth, lead to malformations and in some cases to the death of some corals (Ferreira et al., 2023; Jesus et al., 2022). Furthermore, it has also been reported that they reduce some photosynthetic pigments in algae, leading to their malnutrition (Mao et al., 2018) Finally, it was seen neurotoxicity, malformations, lower heart rate and other alterations in zebrafish embryos (Quintaneiro et al., 2019).

With the ongoing and increasing environmental concern, some natural alternatives to the pollutant synthetic UV filters have been studied.

2.2 Agricultural by-products

Linear economy model consists in a constant supply of products that are used for a short period of time and then discarded. This model leads the unmeasured exploitation of natural resources which has been causing a significant environmental crisis. On the other hand, the circular economy model is a system that values restoration and regeneration of the value of natural sources, limit to an acceptable extent the use of raw materials and energy, and avoid the unnecessary generation of waste. The transition towards the second model is, then, of great importance (Osorio et al., 2021).

The transition towards a circular economy involves different spheres (social, economic and environmental) and generates opportunities for different industries, such as agri-food industry (Osorio et al., 2021).

Agri-food industry consists of using the raw materials from agriculture, fishing, livestock, forestry, and the agri-business and transform them into consumable products. A great part of human diet is constituted of fruits and vegetables. Over the last few decades, it has been discovered that fruits and vegetables have a protective effect over several diseases as well as an antioxidant effect, which lead to intensive marketing campaigns to increase its consumption. With increasing consumption, the amount of waste and residues have also been escalating, reaching around 800 000 tons per year. Another contribution to this high value of waste is the fact that between 10% to 60% raw materials of fruits and vegetable materials is not consumed and is considered waste even though they are, in some cases, more valuable and richer in bioactive compounds than the edible parts. Waste is mainly composed of peels, seeds and leaves and is called “by-products” in order to highlight their potential to be recycled and introduced in different products. Their high bioactive compounds concentration and the need to find alternatives to some synthetic substances has made food by-products an attractive source/material for several industries such as cosmetics (Barbulova et al., 2015).

Onion (*Allium cepa*) peel, an interesting agricultural by-product, comes from the cultivation and consumption of one of the most popular vegetables. Onions are rich in bioactive compounds, mainly alk(en)yl cysteine sulfoxides and flavonoids. Alk(en)yl cysteine sulfoxides are responsible for the flavor of the bulb, while flavonoids determine the color of both the peels and bulb (Celano et al., 2021). The dried peel of onion is a great source of flavonoids (if yellowish) and anthocyanins (in red-colored varieties).

Onion peels are considered inedible and have been less studied and chemical characterized than the edible bulb. Nonetheless, a few studies have classified onion skin a product with a great commercial interest due to its content in flavonoids and other polyphenols, both categories of phenolic compounds.

2.2.1 Phenolic Compounds

Phenolic compounds are plants' secondary metabolites produced during their growth or as a response against environmental stress conditions, and act as a protection for the plant. These compounds possess an extensive range of biological activities due to the different structures they can present, being able to act as antioxidants, anti-inflammatory, anti-allergenic and antimicrobial agents, among others (Grgić et al., 2020).

Although often used as synonyms in literature, polyphenols and phenolic compounds are not exactly the same, as the former term does not include the phenolic compounds containing only one aromatic ring. All phenolic compounds have at least one aromatic ring and one or more hydroxyl groups. Depending on the degree of oxidation, hydroxylation, glycosylation and on interactions with different molecules, phenolic compounds are divided in several groups, as depicted in Figure 2.5 (Grgić et al., 2020).

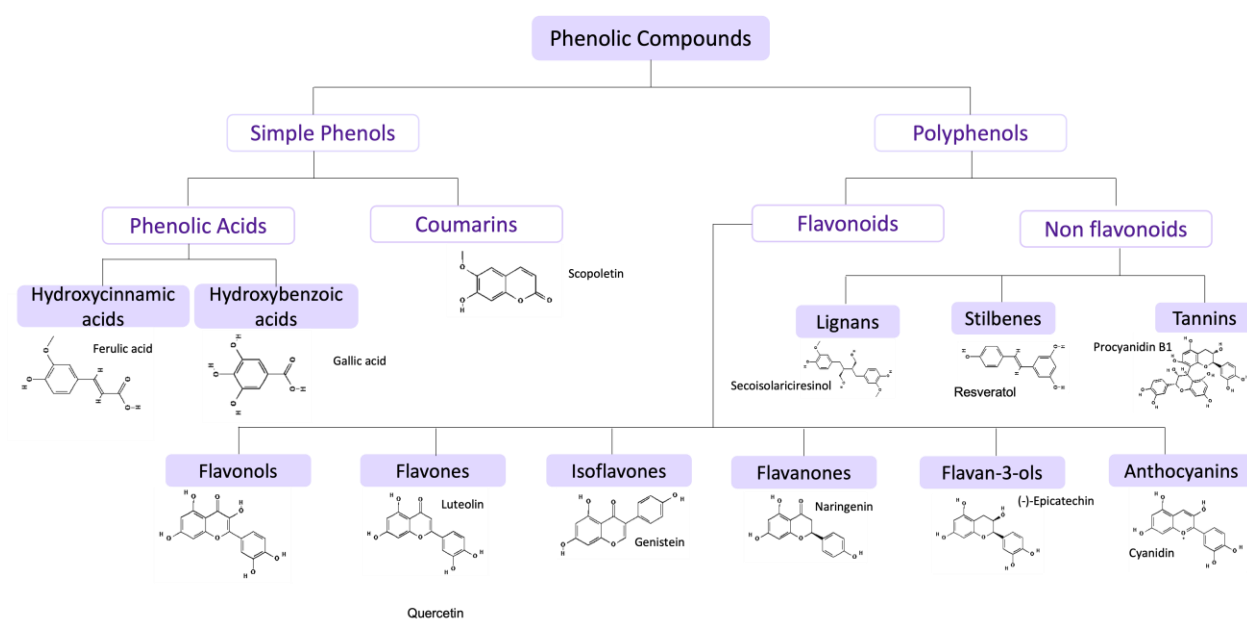


Figure 2.5 - Types of phenolic compounds adapted from (Soto et al., 2015).

Plant extracts are rich in polyphenols causing this group of compounds to be further presented and discussed.

Flavonoids are compounds that present a low molecular mass and a nucleus of two aromatic rings linked by a three-carbon bridge. These molecules protect human cells against damaged induced by free radicals and by short wavelengths radiation, such as UV radiation. There might be a significant number of substitutional groups modifications on the backbone of flavonoids, creating different bioactivities and properties in these compounds which causes a further division in sub-categories of flavonoids (Pinho et al., 2014). The main phenolic compounds in onion peels is a flavonoid, quercetin and its glucosides, which reinforces the interest in the extract from onion peels to be used in sunscreens (Kumar et al., 2022).

Non-flavonoids contain all the other polyphenols, lignans, stilbenes and tannins. In stilbenes group, an interesting compound is resveratrol that has been classified as anti-aging and antioxidant. Resveratrol is another compound significantly present in onion peels which transports these characteristics to the skin of onions (Pinho et al., 2014).

Phenolic compounds all present a strong radical scavenging action and antioxidant properties due to interaction with proteins and ions (Pinho et al., 2014). Due to this fact, it is important to characterize a plant extract according to its antioxidant capacity before incorporating it in a cosmetic product.

The main tests used to characterize the extracts and their description are presented in Table 2.2.

Table 2.2 - Different antioxidant tests and their description.

Test	Description	Advantages	Disadvantages
DPPH (2,2-diphenyl-1-picrylhydrazyl)	Although usually classified as a SET reaction method, DPPH can be neutralized by both electron transfer and H atom transfer. This assay measures the ability of antioxidants to reduce DPPH [•] radical and it is measured by electron spin resonance or by the decrease of the absorbance read.	Easy, simple and fast; Uses only a UV/vis spectrophotometer; It is possible to buy the reagent (DPPH) instead of producing it.	The reaction time is not exact; Due to the DPPH stability and stereo hindrance, the reagent might not be always reactive.
ABTS (2,2'-Azino-bis(3-ethylbenzothiazoline-6-sulfonic acid)	As DPPH, can be neutralized by both reactions, which makes it difficult to conclude about reactivity of the antioxidant, without knowing its composition and structure. ABTS is oxidized to its radical form ABTS ^{•+} , coloured. The antioxidant capacity of the antioxidant is measured by the ability of the sample to decrease the colour of the ABTS ^{•+} radical.	Cheap and easy to perform; Not sensitive to pH; Short reaction time.	Extra step to obtain the reactive form; The free radical is not stable for long periods; It is not a standardized method which may hinder the comparison of results.
FRAP (Ferric Reducing Antioxidant Power)	The FRAP mechanism is based only on electron transfer. By combining this method with different ones, it is possible to distinguish the main mechanism of different antioxidants.	Simple, fast and cost effective; Used globally; No specialized equipment needed.	Might present varying results; Sensitive to temperature and pH.

Source: (Prior et al., 2005)

Antioxidants have two mechanisms of action: Hydrogen Atom Transfer (HAT) and Single Electron Transfer (SET). HAT-based methods determine the ability of an antioxidant to neutralize free radicals by donating hydrogens and the measurements are based on competition kinetics. The reactions in HAT methods are usually fast and solvent and pH independent. A limitation of these methods is that, in the presence of reducing agents, fallacious high apparent reactivity may appear. SET-based methods measure the ability of an antioxidant to reduce any compounds by transference of an electron. These methods are based on deprotonation of the reactive group which makes them pH dependent. They are usually slow methods, which leads to antioxidant capacity being calculated by percentage decrease in product opposing to kinetics calculations (Prior et al., 2005).

Phenolic compounds are easily oxidized, losing their bioactive value, by light, oxygen, or changes in pH or temperature, which creates the necessity to find ways/methods to protect them. A protection method that has been gaining interest from the scientific community is microencapsulation of these compounds.

2.3 Microencapsulation

Nature has long presented natural capsules that protect what is inside of the material from the external environment such as grain, eggshells and cellular membranes. In 1957, it was commercialized the first encapsulated product and ever since, there has been a ceaseless development of different encapsulation methods, whilst its use in several industries has been broadened (Kłosowska et al., 2023).

Encapsulation is defined as a technique in which an encapsulating agent (wall or shell) pack a solid, liquid or gaseous substance (active core material) to elaborate particles with a specific geometry at the nanometer (nanoencapsulation), micrometer (microencapsulation) or millimeter scale (Eghbal et al., 2022). Therefore, encapsulation is then classified according to the particles size as nanoencapsulation (<1.0 μm), microencapsulation (1.0-5000 μm) and macroencapsulation (>5000 μm) (Tarone et al., 2020).

Besides protection, microencapsulation may be used to 1) immobilize the active material, 2) have a controlled release of the core material, 3) increase the stability of the product and 4) extend the shelf life of a product (Kłosowska et al., 2023).

Prior to encapsulating the active agent, it is of utter importance to choose the right coating material in order to achieve the desired properties of the final product. The ideal coating agent must stably emulsify the active agent, not react with the core material, be able to coat the active material (attaining a high encapsulating efficiency) and remain impermeable during processing and long-term storage conditions. There are several available/used coating materials, and these are divided in three main categories according to their chemical properties (Kłosowska et al., 2023; Tarone et al., 2020):

1. Inorganic Polymers;
2. Organic Polymers, natural encapsulants, such as β -cyclodextrin;
3. Inorganic encapsulants.

After selecting the coating material, one must select the microencapsulation technique used, as it also greatly influences the type and shape of the microparticles. The microparticles, classified as either microspheres or microcapsules, can be distinguished based on their internal structure and morphology. Whilst microcapsules can exist in mononuclear or polynuclear forms and are distinguished by the presence of reservoir systems, microspheres are defined by their matrix systems (Paulo & Santos, 2017), as illustrated in Figure 2.6.

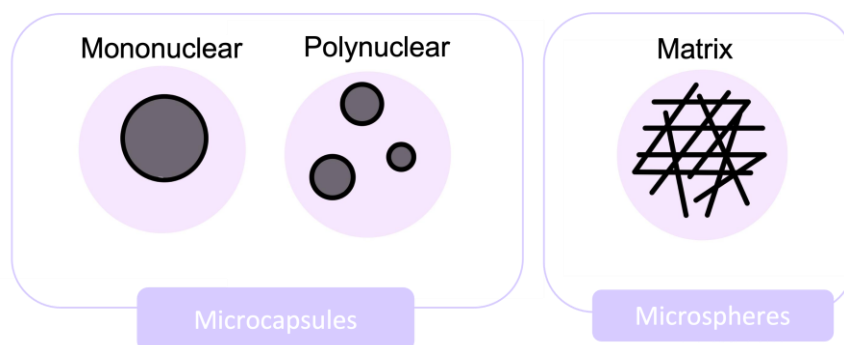


Figure 2.6 - Morphology/shape of particles formed by microencapsulation processes.

The mononuclear morphology refers to microparticles in which a single core is surrounded or trapped by a wall material, while a polynuclear capsule contains two or three cores inside the capsule. Additionally, the matrix morphology is characterized by aggregates of cores embedded in a matrix form, as described by Tarone et al. (2020).

The interaction between the core and the wall materials divides the microencapsulation methods in chemical, physicochemical and mechanical. The main methods of each category are illustrated in Figure 2.7 (Sousa et al., 2022).

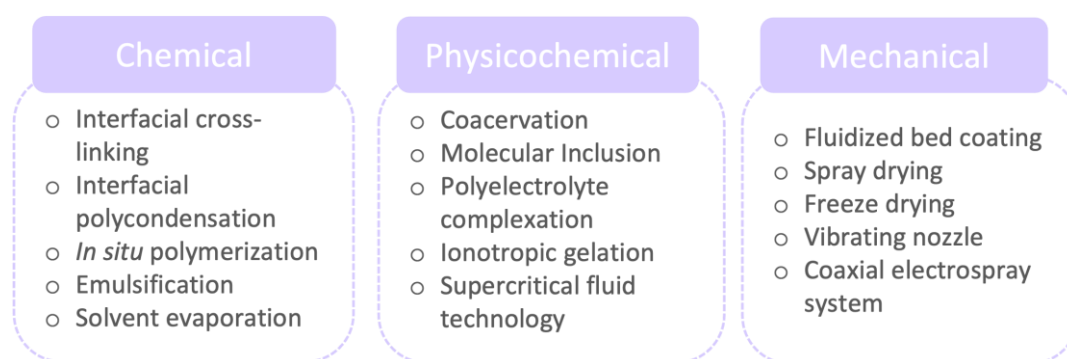


Figure 2.7 - Types of microencapsulation techniques.

Several variables might still influence the formation of the microparticles. Therefore, the microencapsulation method chosen should be optimized to obtain the desired microparticles and outcome. Encapsulation efficiency (EE) and size of the particles are two crucial factors to consider when evaluating a microencapsulation method. EE determines the ability of the wall material to entrap the core agent inside the particle. The particle size is important because it directly affects the delivery properties, solubility and bioavailability of the bioactive compound (Tarone et al., 2020).

The microencapsulation of a wide range of phenolic compounds and plant extracts using various methods has garnered significant interest in recent years. To compile relevant studies, a comprehensive literature search was conducted from 2014 to 2023, utilizing reputable databases such as Scopus, PubMed, and Google Scholar. The search strategy involved incorporating keywords such as “extract”, “encapsulation”, and “phenolic compounds”, leading to the identification and inclusion of pertinent studies in Table 2.4 (summarized version) and in Table A.0.1, in Appendix A.1 (extensive version).

Furthermore, methods used to encapsulate the specific phenolic compounds present in onion peels were evaluated. To compile relevant studies, a comprehensive literature search was conducted from 2014 to 2023, utilizing the same databases mentioned above (Scopus, PubMed, and Google Scholar). The search strategy involved incorporating keywords such as “onion peels”, “encapsulation”, and “phenolic compounds”, leading to the identification and inclusion of pertinent studies in and are presented in Table 2.3.

Table 2.3 - Microencapsulation techniques used for quercetin and resveratrol.

Phenolic compound	Method of encapsulation	Results	Reference
Quercetin	Molecular inclusion with β -CD, hydroxypropyl- β -cyclodextrin (HP- β -CD) and sulfobutyl ether β -cyclodextrin (SBE- β -CD)	Higher scavenging capability than that of quercetin in water and quercetin-SBE- β -CD complex was the most reactive form	(Yousefi et al., 2021)
Quercetin	Molecular complex with β -cyclodextrin (β -CD)	Higher weight loading with a much lower release and also improved its solubility, antioxidant activity, and photostability properties	(Yousefi et al., 2021)
Resveratrol	Molecular complex with β -cyclodextrin (β -CD)	Higher antioxidant capacity	(Pinho et al., 2014)

The main phenolic compounds present in onion peel extracts have been successfully microencapsulated by molecular inclusion using β -cyclodextrin as the encapsulating agent.

Cyclodextrins (CD) are often applied in the pharmaceutical industry as drug carriers to improve solubility, stability and bioavailability of the bioactive compounds. They are approved by the Food and Drug Administration (FDA) and are classified as friendly to the human body. CDs result from the degradation of starch and are saccharides (with 6,7,8 glucose residues) and appear, in the nature, in the α -, β - and γ - forms. The three structures are illustrated in Figure 2.8. Cyclodextrin molecules present a cone shape/format; the inner part of the cone is hydrophobic and the outer surface is hydrophilic, which confers them the ability to form inclusion complexes with poorly water soluble molecules, such as polyphenols (Pinho et al., 2014).

Table 2.4 - Microencapsulation techniques utilized for the encapsulation of several plant extracts.

Encapsulation Method	Core Material	Wall Material	Results	Reference
Hydrogel	Onion peel extract	Soy protein isolate	High EE (97.34%) and increased stability and bio accessibility.	(Stoica et al., 2022)
Freeze drying	Onion Peel extract	β -LG hydrolysates	EE (61.07%) and increased antioxidant capacity	(Milea et al., 2021)
Freeze drying	Onion Peel extract	Maltodextrin	High EE 89.15% and increased thermal stability	(Akdeniz et al., 2018)
Freeze drying	Onion Peel extract	Maltodextrin	High EE 94.30% and higher concentration of polyphenols and antioxidant capacity than the non-encapsulated extract	(Elsebaie & Essa, 2018)
Molecular Inclusion	Blackberry purees	β -cyclodextrin	Increased protection against thermal degradation and higher stability and bio accessibility	(Fernandes et al., 2018)
Freeze-drying	Jaboticaba pomace extract	Maltodextrin, pectin, and soy protein isolate	Protection to monomeric anthocyanin higher than 76% and improved the stability	(Pereira Souza et al., 2017)
Freeze-drying	Blackberry extract	Maltodextrin	EE (76%) and high retention of phenolic content	(Yamashita et al., 2017)
Spray-drying and Molecular Inclusion	Saffron petals extract	β -glucan or β -cyclodextrin	EE 45% and 63.25% respectively; higher bio accessibility in intestine section for both coating agents	(Ahmad et al., 2018)
Spray-drying and Biopolymer Particles	Red grape juice	Soy protein or whey protein / maltodextrin	Higher thermal stability and prolonged shelf life	(Moser et al., 2017)
Double Emulsion and Spray-drying	Black carrot extract	Glycerol mono-oleate and soy lecithin / maltodextrin and poloxamer 338	High protection against heat degradation when subjected to thermal processing and EE 57.2%	(Ravanfar et al., 2018)
Freeze-drying, Complex Coacervation and Molecular Inclusion	Blueberry extract	Maltodextrin, β -Cyclodextrin, Whey protein isolate and Gum Arabic	High protection against heat degradation and EE 82%	(Tao et al., 2017)

EE – Encapsulation Efficiency

The most commonly used is β -CD, as it is the least expensive. β -CD based molecular inclusion complexes are systems that trap a molecule (core material) into the cavity of the β -CDs by hydrogen bonds, van der Waals forces or due to the hydrophobic effect.

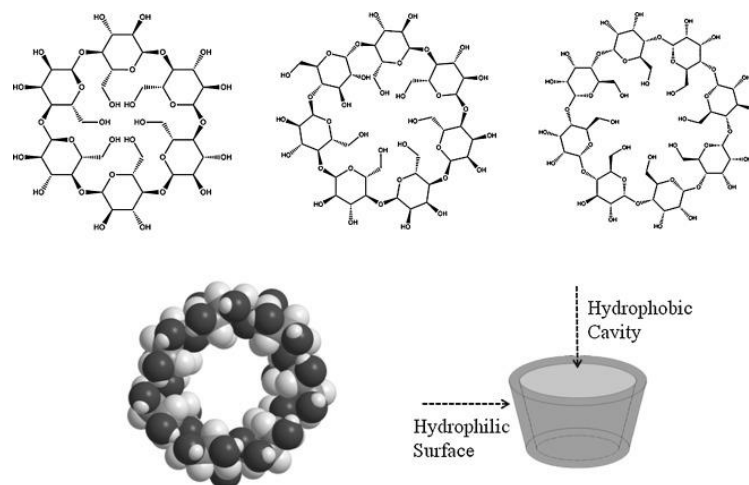


Figure 2.8 – α -, β - and γ - cyclodextrin structures (from left to right) and a 3D representation of a cyclodextrin structure from (Pinho et al., 2014).

β -CDs molecular complexes have been applied in cosmetics in order to (Marques, 2010):

- Increase the water solubility of lipophilic materials;
- Convert liquid or oily materials to powder;
- Improve product stability by protecting the bioactive molecules against decomposition and oxidation;
- Have a controlled released of the active compounds;
- Reduce or prevent skin irritation;
- Control the absorption of specific compounds by the skin;
- Stabilize emulsions and suspensions.

The present study aimed to recover onion peels and the extraction of their phenolic compounds. Afterwards, the study is divided in three parts: (1) characterization of the phenolic extract; (2) extract was microencapsulation by molecular inclusion using β -CD as the coating agent; (3) incorporation of both non-encapsulated and microencapsulated OP extract in produced sunscreen formulations, followed by evaluation of stability and their photoprotection potential to determine whether OP extract might be a possible natural UV filter.

3 Materials and Methods

3.1 Chemical and Reagents

Ethanol (extraction solvent) (Ref. 1.02371.1000, C_2H_6O , CAS 64-17-5) was bought from VWR (Rosny-sous-Bois, France). The reagents used for the antioxidant capacity assays, 2,2-diphenyl-1-picrylhydrazyl (DPPH) (Ref. D9132, $C_{18}H_{12}N_5O_6$, CAS 1898-66-4), 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) (Ref. A1888, $C_{18}H_{24}N_6O_6$, CAS 1898-66-4) and Folin-Ciocalteu reagent (Ref. 47641), were obtained from Sigma Aldrich (St. Louis, MO, USA). In the HPLC analysis, it was used the quercetin standard (Ref. Q4951, $C_{15}H_{10}O_7$, CAS 117-39-5) and resveratrol (Ref. R5010, $C_{14}H_{12}O_3$, CAS 501-36-0) were purchased from Sigma Aldrich (St. Louis, MO, USA); the solvents consisted of ethanol (same reference as above), ultrapure water and acetonitrile (Ref. 83639.320, C_2H_3N , CAS 75-05-8) purchased from Sigma Aldrich (St. Louis, MO, USA). For the Sun Protection Factor calculation, benzophenone-3 (oxybenzone) (Ref. H36206, $C_{14}H_{12}O_3$, CAS 131-57-7) also purchased from Sigma Aldrich (St. Louis, MO, USA). For the microencapsulated process, it was utilized β -cyclodextrin (Ref. W402826, $C_{42}H_{70}O_{35}$, CAS 7585-39-9) was obtained from Sigma Aldrich (St. Louis, MO, USA) and ultrapure water as a solvent. Methanol (Ref. 414816, CH_3OH , CAS 67-56-1) purchased in Carlo Erba (Barcelona, Spain) was used in the assays to characterize the microparticles. For sunscreen formulations products were utilized glycerine (Ref. COSM-01216), xanthan gum (Ref. GOMA-00762), coconut oil (Ref. INCOCO-00185), sweet almond oil (Ref. ACEI-01886), betaine (Ref. COR-COSM-00969) purchased from GranVelada (Zaragoza, Spain) and lecithin (Ref. L0023, CAS 8002-43-5) acquired from TCI (Tokyo, Japan).

3.2 Pre-treatment of the onion peels

Onion Peels used in this work were obtained from white Valencian onions (*Allium cepa* L.) that were cultivated in early April 2022 and harvested at the end of August 2022, in Campo Maior, Portugal. In this culture, only phoskammonium (at the time of the cultivation) and urea (at half of growth time) were used as fertilizers.

Onion peels were dried in a drying oven (P. Selecta, Mod. 210, N. 16472) at 70 °C for 17 hours, and then milled to a diameter inferior to 1 mm. It was used a coffee grinder (Q 5321 Qilive, Auchan, Croix, France) to mill the peels. The resulting particles were stored in a desiccator, at room temperature, covered with paper foil to protect them from light.

3.3 Extraction of the Phenolic Compounds

A solid-liquid extraction with a Soxhlet apparatus was utilized to obtain the OP extract, according to the protocol of Ferreira et al. (2022), with modifications. The solvent used was ethanol, in a mass-solvent ratio of 1:20 m/V. The solvent was poured into a round bottom flask. In this flask, a few boiling regulators were

also added to control the ebullition of ethanol and ensure a steady flow of vapor, to the apparatus operate at maximum efficiency and safely. The sample was inserted in a filter paper cartridge and placed inside a Soxhlet. The Soxhlet was then placed between the round bottom flask and the condenser, in a reflux position. The extraction was performed for 2 hours at a temperature of 70 °C and in triplicate.

The solvent was later evaporated by a rotary evaporator (Rotavapor R-200, BUCHI Laboratories, Flawil, Switzerland) coupled to a Vacuum pump CVC 3000, with a bath temperature of 34 °C and the pressuring varying from 100 to 45 mbar. Finally, the extracts were subjected to a gentle stream of nitrogen to guarantee that all the solvent had evaporated. In order to be considered a dry sample, the mass should not vary more than 1%. Figure C.0.1 in Appendix C.1 illustrates some steps of the extraction procedure.

The extracts were then weighted, and the extraction yield was calculated using Equation (3.1), in which $m_{extract}$ is the mass of the extract after extraction and m_{OP} is the mass of onion peel sample measured prior to extraction.

$$Yield \% = \left(\frac{m_{extract}}{m_{OP}} \right) \cdot 100 \quad (3.1)$$

3.4 Characterization of the Phenolic Extracts

The phenolic extracts were characterized according to several methods in order to determine their antioxidant properties, such as the total phenolic content (TPC) and their radical scavenging activity.

3.4.1 Total Phenolic Content

The Folin-Ciocalteu Method was utilized to determine the TPC of the onion peel extract. In order to proceed with the analysis, it was followed an adapted protocol from literature (Silva et al., 2007).

The results were expressed in gallic acid equivalents (GAE). Hence, it was used an acid gallic calibration curve validated by the research group supervising this project, that was prepared using solutions of varying concentrations (0.5-10 mg L⁻¹) of gallic acid and is presented in Equation (3.2), where Abs is the measured absorbance and C_{GA} (mg_{gallic acid} L⁻¹) is the concentration of gallic acid (Ferreira & Santos, 2022).

$$Abs = 0.0748 \cdot C_{GA} \quad (3.2)$$

The values of the TPC in mg of gallic acid per g of extract (mg_{GAE} g⁻¹_{extract}) were calculated using Equation (3.3), in which C_{sample} (mg L⁻¹) is the extract concentration and Abs_{sample} the measured absorbance.

$$TPC = \frac{\left(\frac{Abs_{sample}}{0.0748} \right)}{C_{sample}} \cdot 1000 \quad (3.3)$$

The analyzed sample consisted of a solution with the concentration of 1000 mg L⁻¹.

Firstly, 20 µL of the sample were added to a 2 mL cuvette, along with 100 µL of Folin-Ciocalteu reagent and 1580 µL of water. After 3 to 6 minutes, 300 µL of sodium carbonate solution, with a concentration of

333.3 g L⁻¹ (saturated solution), were added to the cuvette and the resulting solution was incubated for 2 hours in the dark, at room temperature. After the incubation period, the absorbance of the solution was determined using a Thermo GENESYS 10S UV-Vis spectrophotometer, at a wavelength of 750 nm.

3.4.2 Antioxidant Capacity of the Onion Peel Extracts by the DPPH assay

An assay with the radical 2,2-diphenyl-1-picrylhydrazyl (DPPH) was performed, in order to evaluate the Antioxidant Capacity of the OP extract. The assay was performed by applying the microplate method as described by Bobo-García et al. (2015).

The results were expressed in Trolox equivalents (TE). Hence, a Trolox calibration curve, based on the percentage of DPPH inhibition of Trolox standards, was prepared with concentration between 25 and 250 mg L⁻¹, as presented in Equation (B.1.1) and in Figure B.0.2 in Appendix B.1. A DPPH inhibition curve for OPE was also developed. The extract solutions were prepared in ethanol in a concentration range of 75 to 750 mg L⁻¹.

When performing the assay, 20 µL of the sample/Trolox standard were made react with 180 µL of DPPH solution, at a concentration of 150 µmol L⁻¹, and left to incubate in the dark for 40 minutes. Then, the absorbance was read at 515 nm in the computer software Gen5 (Agilent Bio Tek). As a blank it was utilized a solution consisting of 20 µL of water and 180 µL of ethanol and as a control it was added 20 µL of water to 180 µL of DPPH solution.

The percentage of DPPH inhibition was calculated through Equation (3.4), where $Abs_{control}$ refers to the absorbance of the control, Abs_{blank} refers to the absorbance of the blank and Abs_{sample} is the measured absorbance for the sample solutions.

$$DPPH \text{ inhibition } \% = \left(1 - \frac{Abs_{sample} - Abs_{blank}}{Abs_{control} - Abs_{blank}} \right) \cdot 100 \quad (3.4)$$

The concentration of extract that is necessary to inhibit 50% of DPPH (IC₅₀) was also calculated as stated in Equation (B.1.2) in Appendix A.1.

3.4.3 Antioxidant Capacity of the Onion Peel Extracts by the ABTS assay

To further evaluate the antioxidant capacity of OP extract, an assay was performed with the 2,2'-Azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) ABTS radical, commonly known as Trolox Equivalent Antioxidant Capacity (TEAC), following the Xiao et al. (2020) protocol.

To express the results in TEAC, a Trolox calibration curve was prepared, and it is presented in Equation (B.2.1) and Figure B.0.4 in Appendix B.2. The Trolox standards varied in a concentration range of 10 to 150 mg L⁻¹.

To perform the assay, OP extract solutions were prepared in ethanol with the concentration varying from 20 to 150 mg L⁻¹. 20 µL of these solutions, or the Trolox standard solutions, were added to 180 µL of ABTS solution, and were then incubated in the dark for 15 minutes. At the end, the absorbance was read at 734

nm in the computer software Gen5 (Agilent Bio Tek). It was utilized a solution of 20 μL of 0.05 M acetic acid buffer solution (pH 4.6) and 180 μL of ABTS solution as a control.

The percentage of ABTS inhibition was calculated using Equation (3.5), where $Abs_{control}$ refers to the absorbance of the control and Abs_{sample} is the measured absorbance for the sample solutions.

$$ABTS \text{ inhibition } \% = \left(\frac{Abs_{control} - Abs_{sample}}{Abs_{control}} \right) \cdot 100 \quad (3.5)$$

3.4.4 Identification and quantification of Phenolic Compounds in the OP extract by HPLC

The phenolic compounds present in the OP extract were quantified by high-performance liquid chromatography with a diode array detector (HPLC-DAD). The analyses were performed using an Elite LaChrom (Hitachi, Japan) HPLC system, that was equipped with a Hitachi L-2200 autosampler L-2130 pump and a L-2455 diode array detector. The solvent used to dissolve the samples was a mixture of acetonitrile, water and ethanol in the ratio of 2:1:1 V/V/V. Samples were injected in a Puroshper STAR RP-18 LiChroCART chromatography column (Merck, Germany) and were used Milli-Q water with 0.5% of orthophosphoric acid and methanol:acetonitrile (80:20 V/V) as phase A and B, respectively.

The phenolic compounds were quantified by the external standard method as presented by Ferreira et al. (2023) and that is described in Appendix C.1.

3.4.5 Sun Protection Factor

The photoprotection capacity of the onion peel extract was determined by calculating the Sun Protecting Factor (SPF) following the Mukund Manikrao and Sharada Laxman (2016) protocol, with slight modifications.

Firstly, solutions of OPE, with a concentration of 0.01, 0.045 and 0.095 mg mL^{-1} , were prepared, as well as a 0.01 mg mL^{-1} solution of (2-Hydroxy-4-methoxyphenyl)-phenylmethanone (oxybenzone), to be used as a positive control for a comparison between the performance of the OP extract with a synthetic UV filter. Oxybenzone was chosen as positive control as it is one of the synthetic UV most used in commercial sunscreens. The solvent used was ethanol for all solutions.

The absorptions values of the samples were then measured in a UV-3100PC spectrophotometer (VWR, Darmstadt, Germany) in the wavelength range of 280 to 400 nm, in a 1 cm optical path length quartz cell. The SPF values were calculated using Equation (3.6), in which CF is the correction factor equal to 10, $EE(\lambda)$ refers to the erythemal effect spectrum, $I(\lambda)$ is the solar intensity spectrum for a specific wavelength λ , and $EE(\lambda) \times I(\lambda)$ represents the normalized product function, and its values are presented in Table A.1.0.1 in Annex A.1. $Abs(\lambda)$ refers to the absorbance value of the sample at the wavelength λ .

$$SPF = CF \cdot \sum_{290}^{320} EE(\lambda) \cdot I(\lambda) \cdot Abs(\lambda) \quad (3.6)$$

3.5 Microencapsulation

The microencapsulation of the OP extract was performed according to the protocol followed by Kalogeropoulos et al. (2010), with some modifications. Firstly, OP extract and β -CD (beta-cyclodextrin) were solubilized in water, using the ratio of 1 g of extract to 4 g of β -CD solubilized in 250 mL of water. The solution was, then, put in an ultrasound bath for 5 min, followed by a period of 2 hr of magnetic stirring at 1000 rpm. Furthermore, the solution was subjected to a high-performance homogenizer, Ultra-Turax (IKA T18 Digital ULTRA-TURAX), during 5 min at a velocity of 5000 rpm. Finally, the obtained solution was vacuum filtered, using 0.20 μ m PVDF (Polyvinylidene difluoride) filters. Figure D.0.1 in Appendix D.1 illustrates some steps of the microencapsulation procedure.

The filters containing the microparticles were frozen at -80°C for 18 hr and then, freeze dried for 24 hr, to ensure that there was no water present in the microcapsules. The resulting microparticles were stored in a desiccator, at room temperature, covered with paper foil to protect them from light.

3.5.1 Characterization of Microcapsules

3.5.1.1 Particle Size Distribution

The particle size distribution and polydispersity were evaluated in triplicate. The analyses were performed by a Coulter Counter-LS 230 Particle Size Analyzer (Miami, FL, USA) equipment. The results are presented considering the average values.

The polydispersity of particle size distributions was evaluated according to the polydispersity index (PDI), using Equation (3.7) (Paulo & Santos, 2020).

$$PDI = \left(\frac{D_{v,90} - D_{v,10}}{D_{v,50}} \right) \quad (3.7)$$

$D_{v,90}$ represents the maximum particle diameter below which 90% of the sample volume exists, $D_{v,10}$ is the maximum particle diameter below which 10% of the sample volume exists, and $D_{v,50}$ consists of the maximum particle diameter below which 50% of the sample volume exists.

3.5.1.2 Production Yield and Encapsulation Efficiency

The production yield (PY) is the ratio between the mass of obtained freeze-dried microparticles, m_f , and the amount of polymer and extract used, m_o , and is calculated through Equation (3.8).

$$PY \% = \left(\frac{m_f}{m_o} \right) \cdot 100 \quad (3.8)$$

To calculate the encapsulation efficiency (EE), the microcapsules were destroyed to release the encapsulated extract, which was accomplished by following a modified protocol presented by da Rosa et al. (2014). A solution of 10 mg of powder was prepared in 4 mL of methanol and was put in a ultrasounds bath for 5 min, followed by 10 min of centrifugation with an acceleration of 1502 g. The supernatant was

filtered through 0.20 μm PVDF filters and the filtered is, then, subjected to a gentle stream of nitrogen to evaporate the methanol. The obtained solid was solubilized in ethanol and analyzed via spectrophotometry.

The EE is calculated according to Equation (3.9), where m_b is the initial amount of extract used to produce 10 mg of microparticles and m_a is the amount of extract detected in the ethanolic solution after the microparticles destruction.

$$EE \% = \left(\frac{m_a}{m_b} \right) \cdot 100 \quad (3.9)$$

In order to obtain the mass of extract in the ethanolic solution, it was prepared a calibration curve of ethanolic solutions of OP extract, with concentrations varying from 0.010 mg mL^{-1} to 0.1 mg mL^{-1} , by reading their absorbance at 290 nm on the UV-vis spectrophotometer, using ethanol as blank. The wavelength of 290 nm was chosen because, after analyzing several spectra of ethanolic solutions of OPE, it was seen that the maximum absorbance was measured for the wavelength of 290 nm. The resulting equation is presented in Equation 3.10 and illustrated in Figure D.0.2 in Appendix D.2. The absorbance (Abs) of the final ethanolic solution was measured at 290 nm and using Equation 3.8 it was calculated the concentration of OP extract ($C_{extract}$) (mg mL^{-1}) in the solution. By multiplying this value by the volume of added ethanol, the mass of extract in the ethanolic solution was obtained.

$$Abs = (9.73 \pm 0.33) \cdot C_{extract} - (0.02 \pm 0.02) \quad (3.10)$$

3.5.1.3 Antioxidant capacity

The antioxidant capacity of the microencapsulated extract was determined by the ABTS and DPPH assays, following the same protocols as described in Sections 3.3.2 and 3.3.3, using methanol as a solvent instead of ethanol. Similarly to the quantification of the encapsulation efficiency, the protocol detailed above was followed to the filtration step. The filtered was considered the methanolic base solution. Several dilutions were performed to obtain the solutions needed to perform the assays. Solutions of OPE of the same concentrations were made to compare results. As the results were presented in Trolox equivalents, a calibration curve was also performed.

3.5.1.4 Morphology

The morphological structures of microparticles were analyzed by scanning electron microscope (SEM) analysis using a Tescan VEGA LMS equipment. The samples were previously coated of gold palladium alloy that was applied using an Agar Sputter Coater equipment. The SEM analysis was conducted at an accelerating voltage of 30 keV with a magnification of 100x.

3.5.1.5 Thermal Properties

The thermal properties of the microparticles were assessed using thermogravimetric analysis (TGA) and differential scanning calorimetry (DSC) with the aid of Hitachi STA300 and Shimadzu DSC-60 instruments, respectively. In the TGA analysis, each sample weighting approximately 10 mg was subjected

to heating from 25 °C to 550 °C at a constant rate of 10 °C min⁻¹. For the DSC analysis, the samples were placed in sealed aluminum pans and heated from 25 °C to 11 °C at a rate of 10 °C min⁻¹.

3.6 Sunscreen Formulations

In order to evaluate the effect of OPE and its behavior as a UV filter (non-encapsulated and encapsulated) in a cosmetic formulation, five different O/W emulsions were produced. The different used ingredients, as well as their concentration in the formulation, are presented in Table 3.1. The base formulation consisted of 2 phases: phase A (aqueous phase) containing ultrapure water, glycerin and xanthan gum and phase B (oily phase) made of coconut oil, sweet almond oil, lecithin and betaine. Both phases were put in a heating bath at 70 °C, separately, and blended until total mixture. Then, phase B was added to phase A while maintaining the stirring and temperature. The final mixture was homogenized with an IKA T18 Digital ULTRA-TURAX for 2 min at 12 000 rpm. The formulations were cooled until it reached 40 °C and the stabilizers were added to the creams, except for negative control, while stirring to achieve homogenization. Figure E.0.1 in Appendix E.1 illustrates the steps of production of the base formulation.

The first formulation consisted of the base formulation and was used as a negative control (NC). A synthetic UV filter (oxybenzone) was added to the second formulation and was used as positive control (PC). OPE (non-encapsulated) was added to the third formulation, microencapsulated OPE (MP-OPE) was added to the fourth formulation and, finally, a mixture of the 3 stabilizers (MIX) was used in the final formulation. Regulations state that the maximum concentration of oxybenzone in a cosmetic formulation cannot surpass 10%, which was respected in all formulations (Parliament, 2009).

Table 3.1 - Concentration of the several ingredients used in the sunscreen formulations.

Phase	Ingredients	Function	NC / g	PC / g	OPE / g	MP-OPE / g	MIX / g
Phase A	Water	Solvent	73.6	73.6	73.6	73.6	73.6
	Glycerin	Humectant	7.6	7.6	7.6	7.6	7.6
	Xanthan Gum	Thickener	0.6	0.6	0.6	0.6	0.6
Phase B	Coconut oil	Emollient	7.6	7.6	7.6	7.6	7.6
	Sweet Almond oil	Emollient	6.8	6.8	6.8	6.8	6.8
	Betaine	Emulsifier	2.7	2.7	2.7	2.7	2.7
	Lecithin	Secondary emulsifier	1.1	1.1	1.1	1.1	1.1
Stabilizers	Oxybenzone	Synthetic UV filter	-	5.0	-	-	1.6
	OP extract	Natural antioxidant and UV filter	-	-	5.0	-	1.6
	MP-OP	Natural antioxidant and UV filter	-	-	-	5.0	1.6

NC – negative control formulation; PC - positive control formulation; OPE – Onion Peel Extract formulation; MP-OPE – Microencapsulated Onion Peel Extract formulation; MIX – Mixture formulation; OP – Onion Peel; MP-OP – Microencapsulated Onion Peel extract.

3.6.1 Stability Tests

In order to ensure the stability of the formulations, the following tests were performed 2/3 days after production (t0), two weeks after (t1) and four weeks after production (t2). All tests, except for the thermal stability test, were performed at room temperature, following the protocols present in Ferreira et al. (2023).

3.6.1.1 pH Test

1 g of each formulation was dissolved in 4.5 mL of purified water and its pH was measured using a digital pH meter. The sample was kept under stirring for the test.

3.6.1.2 Centrifugation Test

Each formulation was evaluated regarding its physical stability by being subjected to 10 min of centrifugation at 2670 g. Physical stability was evaluated by visually verifying if phase separation and color changes occurred.

3.6.1.3 Thermal Stability Test

Heat-cold-heat cycles were used to study the thermal stability of formulations. Formulations were incubated at 50°C for 24 hr, then stored at 4°C during 24 hr, followed by 24 hr of incubation at 50°C and, finally, 24 hr at 4°C to complete the cycle, based on a modified protocol by R. C. Ribeiro et al. (2015). Thermal stability was evaluated by mass changes and, visually, by verifying textural and color consistency.

3.6.2 Antioxidant Capacity

To study the effect of OPE in the antioxidant capacity of the sunscreen, the phenolic compounds were extracted from the formulations. 2 g of sunscreen formulations were added to 4 mL of ethanol. The solution was homogenized for 1 min in a vortex, placed in a ultrasounds bath for 5 min and centrifuged at 1502 g for 5 min. The supernatant was collected and stored. 4 mL of ethanol were to the formulation and the cycle described before was repeated. The supernatants were homogenized and used to perform the DPPH assay. The DPPH assay was performed following the protocol described in Section 3.3.2.

3.6.3 Sun Protection Factor

To evaluate the performance of OPE as a natural UV filter and compare its performance against a synthetic UV filter, it was carried out the evaluation of the sun protection factor of the formulations in accordance with the protocol presented in Section 3.3.4.

3.6.4 Photoprotection evaluation – skin reactivity

All the formulations were applied to the inner arm of a volunteer in a marked area. The volunteer was exposed to sunlight for one hour. The skin reaction was visually evaluated a few hours after exposure. To assess the skin reaction, a photograph was taken before and 3 hours after sun exposure.

3.7 Statistical Analysis

In order to compare the obtained results, a statistical one-way analysis of variance (ANOVA) was performed by calculating the p-value (95% confidence), with p-values below 0.05 indicating no significant difference between extraction times.

3.8 Treatment of waste

The waste generated was mainly liquid solutions of organic solvents such as ethanol, acetone and methanol. The residues were collected in closed containers, labelled in accordance with the norms, and stored away from light and potential ignition sources, until EcoFEUP (FEUP's Environmental Management System) was able to proceed with the waste treatment.

4 Results and discussion

4.1 Extraction yield and Characterization of the Onion Peels Extract

The onion peel extract was obtained through solid-liquid extraction, using a Soxhlet apparatus, with a production yield of $(15.72 \pm 1.79)\%$. Table 4.1 presents the results for the characterization of the Onion Peels extract.

Table 4.1 - Obtained results for the characterization of the onion peels extracts.

	Test	Mean $\pm \sigma$
DPPH	TPC / $\text{mg}_{\text{GAE}}\text{g}^{-1}\text{extract}$	585.23 ± 10.43
	TE / $\text{mg}_{\text{TE}}\text{g}^{-1}\text{extract}$	199.71 ± 1.16
	IC ₅₀ / mgL^{-1}	31.01 ± 1.98
	ABTS / $\text{mg}_{\text{TEAC}}\text{g}^{-1}\text{extract}$	381.45 ± 8.05
HPLC-DAD	Quercetin / $\text{mgg}^{-1}\text{dried extract}$	4.48 ± 0.01
	Resveratrol / $\text{mgg}^{-1}\text{dried extract}$	0.51 ± 0.02

The results are expressed as mean $\pm$ standard deviations of 4 independent measurements. TPC: total phenolic content; GAE: gallic acid equivalents; IC₅₀: the concentration of extract necessary to inhibit 50% of DPPH radical; TE: Trolox equivalents; TEAC: Trolox equivalent antioxidant capacity.

The TPC value obtained is within the range of the literature values, but it is closer to the higher values of the literature, as presented in Table 4.2, demonstrating a high phenolic content in this extract.

Furthermore, the antioxidant capacity of the OP extract was also evaluated by the DPPH assay and ABTS assay. The results vary depending on the extraction method, solvent used and, also, on the variety of onion utilized. Furthermore, to perform these assays, small volumes are utilized, which may lead to significant errors and may cause the results to vary significantly between authors. The obtained results appear in the range of the literature; however, are slightly lower than those obtained for the same extraction method, presented on the first line of Table 4.2.

It is also important to evidence the value of the half maximal inhibitory concentration (IC₅₀), which represents the concentration of extract needed to inhibit 50% of the DPPH present in solution. The obtained value is inferior to 50 mgL^{-1} , which means this extract is considered a very strong antioxidant (Fitriyasyah et al., 2021).

Quercetin and resveratrol were the found to be the main phenolic compounds present in the onion peels extract, which is in accordance with the literature. Literature also refers the presence of Kaempferol; however, this compound was not detected in the studied OP extract (Ferreira et al., 2023).

Table 4.2 - Comparison of antioxidant capacity values of OP extract from the literature.

Study	Extraction Method	TPC / $\text{mg}_{\text{GAE}} \text{g}^{-1} \text{extract}$	DPPH / $\text{mg}_{\text{TE}} \text{g}^{-1} \text{extract}$	ABTS / $\text{mg}_{\text{TEAC}} \text{g}^{-1} \text{extract}$
(Ferreira et al., 2023)	Solid-liquid extraction using a Soxhlet apparatus for 1.5 h	374.4 ± 16.4	308.9 ± 2.8	413.4 ± 22.5
Bordin Viera et al. (2023)	Ultrasound-assisted extraction using a Sonic Vibra-Cell ultrasound generator operating at 20 KHz	117.50-822.87	6.54-14.93	2.36-19.67
Benito-Román et al. (2020)	Incubator shaker with ethanol:water (70:30 V/V) for 60 min at 37 °C	46.7 ± 1.4	-	-
Akdeniz et al. (2018)	Water bath at 40 °C shaking at 70 rpm for 4 h, using ethanol:water (50:50 V/V)	510.87 ± 4.04	-	-
Lee et al. (2014)	Water bath at 60 °C for 3 h, using ethanol as solvent	372.50 ± 6.85	77.15 ± 2.74	-

The results are expressed as mean $\pm$ standard deviations. TPC: total phenolic content; GAE: gallic acid equivalents; TE: Trolox equivalents; TEAC: Trolox equivalent antioxidant capacity

4.1.1 Sun Protection Factor

The sun protection factor (SPF) is the numerical indicator of the protection level of a sunscreen against UV rays which damage the skin. The higher the SPF of a product, the more effective it is at preventing sunburn.

The sun protection factor was analysed for different concentrations of onion peel (OP) extract and was compared to a synthetic UV filter serving as positive control (oxybenzone), as presented in Table 4.3. The absorbance spectra are illustrated in Figure 4.1.

Table 4.3 - SPF values for solutions of OP extract at different concentrations and for a solution of oxybenzone.

Sample	Concentration / mg mL^{-1}	SPF
Oxybenzone (positive control)	0.010	38.84
Onion Peel Extract (OP)	0.010	6.05
Onion Peel Extract (OP)	0.045	24.92
Onion Peel Extract (OP)	0.095	60.24

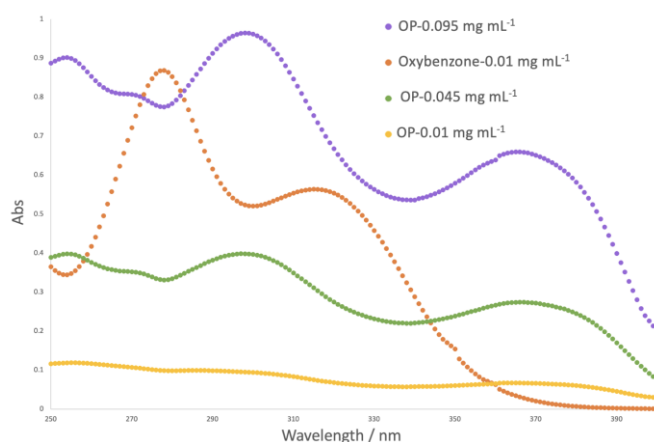


Figure 4.1 - Absorbance spectra of solutions of oxybenzone and Onion Peel (OP) extract at different concentrations.

The results demonstrate that, for the same concentration, OP extract solution presents a smaller SPF value than the solution of the synthetic UV filter. The concentration of synthetic UV filter that might be used in a cosmetic formulation is limited by legislation. Nevertheless, OP extract is a mixture of natural compounds (instead of a specific compound as the synthetic UV filter), and so, might be used in higher concentrations. The results reveal that for higher concentration, solutions of onion peel extract are able to achieve higher SPF values than the synthetic UV filter, which demonstrates that OP extract presents a high potential as natural UV filter.

Furthermore, Figure 4.1, shows that OP absorbs in the same UV region as oxybenzone. OP extract also absorbs radiation at higher values of wavelength (350-390 nm), in which oxybenzone does not absorb, possibly providing protection against a wider range of UV radiation than the synthetic compound.

4.2 Microencapsulation

Microencapsulation by molecular inclusion, using β -cyclodextrin as the coating polymer, was used, after some modification of the protocol presented by Kalogeropoulos et al. (2010). The first protocol followed was similar to the one presented in Section 3.4, using 0.45 μm PVDF filters instead of 0.2 μm filters. A Coulter analysis detected several particles with a diameter inferior to 0.4 μm , as shown in Figure 4.2. In addition to the observation of a strong orange colour in the obtained filtered sample. These observations suggest that a considerable number of microparticles may have passed through the filter, resulting in sample loss and decreased production yield, an inference that was confirmed by an ABTS assay similar to the one described in Section 3.3.3.

This assay was performed 24 hr after the vacuum filtration, allowing the release of the extract contained in microcapsules. The concentration of the filtered was estimated based on the assumption that the encapsulation efficiency was 80%. Dilutions were made to achieve all the necessary solutions. Onion peel solutions of the same concentrations were prepared to compare and confirm the results. Most filtered solutions inhibited 100% of the ABTS agent, whereas OP extract with the highest concentration only inhibited 80% of the ABTS radical. In conclusion, the filtered sample was determined to hold more than the anticipated 20% of the OP extract, thus verifying the release of the extract contained within the microparticles as they passed through the filters.

In the protocols further followed, 0.2 μm PDVF filters were used to avoid this issue. As referred in the state of art, microparticles' diameter is within the range of 1 to 5000 μm . To increment the diameter of the particles, slight modifications were made to the protocol.

Thereafter, two different protocols were studied. The initial protocol involved homogenizing the solution at a velocity of 5000 rpm for 5 minutes, which was reduced to 3 minutes, while the latter protocol required homogenization of the solution at a velocity of 3000 rpm for 5 minutes. A Coulter analysis was performed to determine the particles size distribution, as presented in Figure 4.2. The production yield and the polydispersity are presented in Table 4.4.

Table 4.4 - Production yield and polydispersity of the first 3 protocols.

Sample	PY / %	D _{V,10}	D _{V,50}	D _{V,90}	PDI	Mean Diameter / μm
5000 rpm/5min	6.75	2.39	29.27	69.42	2.29	32.30 $\pm$ 26.02 ^a
3000 rpm/5 min	11.97	3.43	13.46	33.82	2.25	16.23 $\pm$ 11.80 ^a
5000 rpm/3 min	9.93	4.91	41.79	508.20	12.04	163.1 $\pm$ 215. ^a

PY % - Product yield; PDI – Polydiversity Index. Different lowercase letters (a–c) in the same column represent statistically different values ($p < 0.05$) between each sample.

The production yield presents a small value, as expected for encapsulation using inorganic polymers as coating agents, and molecular inclusion as the encapsulation method (Ravindran Maniam et al., 2022).

In relation to the particle size, the second protocol decreases the particles diameter in comparison to the original protocol. The last protocol increases the diameter of the particles, but provokes large polydiversity, contrary to the desired.

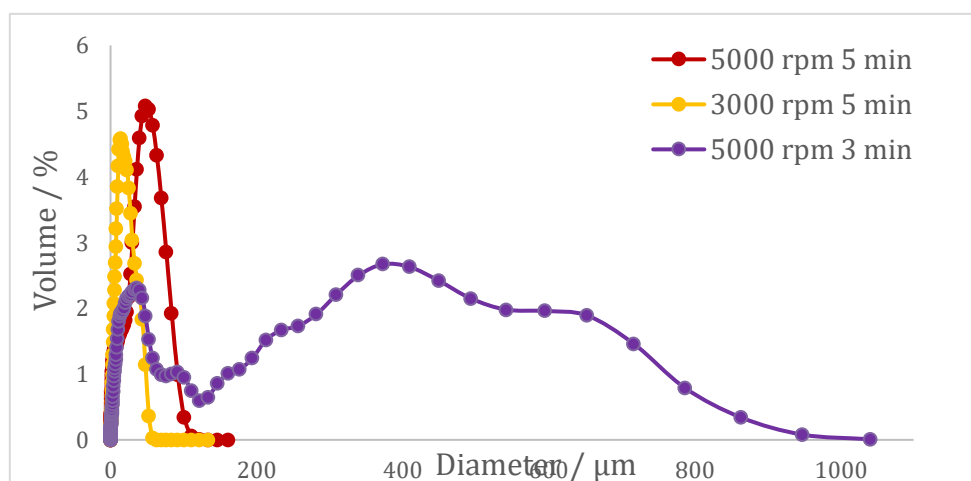


Figure 4.2 - Particle size distribution of several sample of microparticles.

Considering both the mean diameter of the particles and the polydiversity obtained, it was chosen to follow with the original protocol, but recurring to 0.2 μm PDVF filters.

The following step in the optimization process consisted in studying the effect of the loading capacity in the microparticles. The loading capacity is the amount of encapsulated material per unit weight of microparticle. Therefore, the protocol was followed for different ratios of mass of extract/mass of β -cyclodextrin, 1 g:4 g, 1 g:6 g and 1 g:10 g.

The obtained powders were evaluated regarding their particle size distribution, their polydispersity, their antioxidant capacity and the encapsulation efficiency, as described in the Section 3.4.

The particle size distribution results are illustrated in Figure 4.3. The results regarding the product yield, polydispersity and encapsulated efficiency for each of the obtained powders are presented in Table 4.5. The sample with a ratio of 1 g:10 g encapsulated an insufficient amount of extract, causing the Coulter equipment to be incapable of analysing it, and as a result, no values of mean diameter or polydispersity were obtainable.

The samples 1:4 and 1:6 exhibit a bimodal distribution of particle sizes, suggesting the formation of aggregates of microparticles during the batch production, as illustrated in Figure 4.3. The mean diameter values obtained for sample 1:4 are found to be higher than the values obtained for sample 1:6, measuring 30.84 μm and 21.30 μm , respectively. This difference can be associated with the higher concentration of material verified in the sample 1.4. According to Tavares et al. (2021), the mean diameters of powders can be influenced by various factors, such as the nature of wall materials, the type of core material, concentrations of wall materials, stirring speed, and polymer molecular weight. For the Sample 1:4 and 1:6, the PDI values are higher than 1. These findings indicate that the samples exhibit a wide range of particle sizes and a greater degree of heterogeneity (Tavares et al., 2021; Zapata Noreña et al., 2015). The higher PDI value obtained for sample 1:6 indicate a greater degree of heterogeneity in the particle size distribution. The higher PDI value obtained for sample 1:6, compared to sample 1:4, suggests a greater degree of heterogeneity in the particle size distribution. This indicates that the particles in sample 1:6 vary to a larger extent in terms of their sizes, resulting in a broader size distribution compared to sample 1:4.

Table 4.5 - Product yield, polydispersity and encapsulation efficiency of particles with different ratios of compounds.

Sample / g:g	PY / %	D _{V,10}	D _{V,50}	D _{V,90}	PDI	Mean Diameter / μm	EE / %
1:4	8.29	4.18	27.77	64.71	2.09	30.84 $\pm$ 22.14 ^a	91.80 $\pm$ 0.03 ^a
1:6	5.91	3.73	17.81	43.52	2.23	21.30 $\pm$ 16.03 ^a	72.03 $\pm$ 0.05 ^b
1:10	7.78	-	-	-	-	-	19.71 $\pm$ 0.04 ^c

PY % - Product yield; PDI – Polydiversity Index. EE – Encapsulation Efficiency. Different lowercase letters (a–c) in the same column represent statistically different values ($p < 0.05$) between each sample.

Considering their mean diameter and their polydispersity, particles with a ratio of 1 g of extract to 4 g of polymer apparently represent the optimal choice.

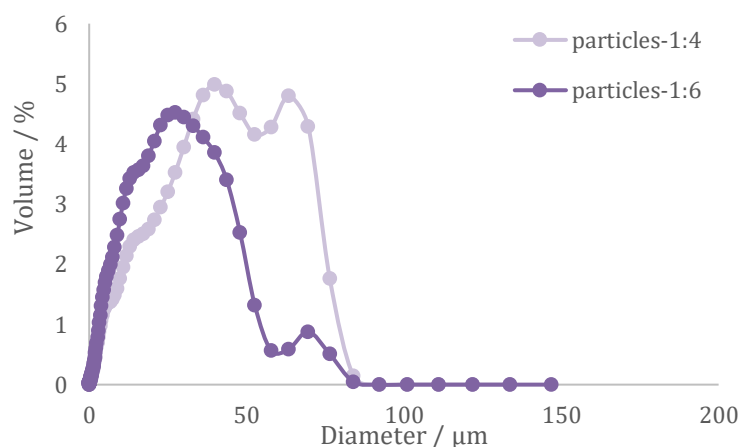


Figure 4.3- Particle size distribution of the samples produced, 1 g_{extract}:4 g_{coating agent} and 1 g_{extract}:6 g_{coating agent}.

Encapsulation efficiency measures the amount of extract that was successfully entrapped in the microcapsule and is one of the most important characteristics when evaluating encapsulation processes. This polymer is more efficient in the encapsulation process when presenting a high loading capacity.

Ultimately, the antioxidant capacity of the microencapsulated extract was assessed and subsequently compared with that of the non-encapsulated extract. The results are present in Table 4.6. The sample of the ratio 1 g:10 g did not present any antioxidant capacity, not being able to inhibit the DPPH radical.

Table 4.6 - Antioxidant capacity of the different sample by the DPPH assay.

Sample / $g_{\text{extract}} : g_{\text{coating agent}}$		TE / $mg_{\text{extract}} g_{\text{Trolox}}^{-1}$	IC ₅₀ / $mg L^{-1}$
1:4	Encapsulated	303.76 ± 58.56^a	23.66 ± 4.10^a
	Non-encapsulated	115.78 ± 6.00^b	60.67 ± 3.05^b
1:6	Encapsulated	178.89 ± 15.92^c	41.04 ± 3.46^b
	Non-encapsulated	129.11 ± 7.90^b	56.87 ± 3.47^b

The results are expressed as mean $\pm$ standard deviations of 4 independent measurements. IC₅₀: the concentration of extract necessary to inhibit 50% of DPPH radical; TE: Trolox equivalents. Different lowercase letters (a–c) in the same column represent statistically different values ($p < 0.05$) between each sample.

Analysing the results of the DPPH assay revealed that the microencapsulated extract exhibits superior antioxidant capacity in comparison to the non-microencapsulated extract, a finding that is consistent with the existing literature (Pinho et al., 2014). Moreover, the sample with the ratio of 1 g_{extract} to 4 $g_{\text{coating agent}}$ demonstrates better antioxidant capacity than the sample with the ratio of 1 g_{extract} to 6 $g_{\text{coating agent}}$.

Following careful consideration and analysis of the outcomes obtained from all the carried out assays, it can be deduced that the optimal protocol is a variation of the original technique that employs 0.2 μm PDVF filters. This particular method will be employed in the microencapsulation of the extract for incorporation in sunscreens.

4.2.1 Microparticles morphology

Both OP extract microparticles and the β -CD powder (coating agent) analyzed by scanning electron microscopy (SEM), in order to examine their morphology. The results are illustrated in Figure 4.4.

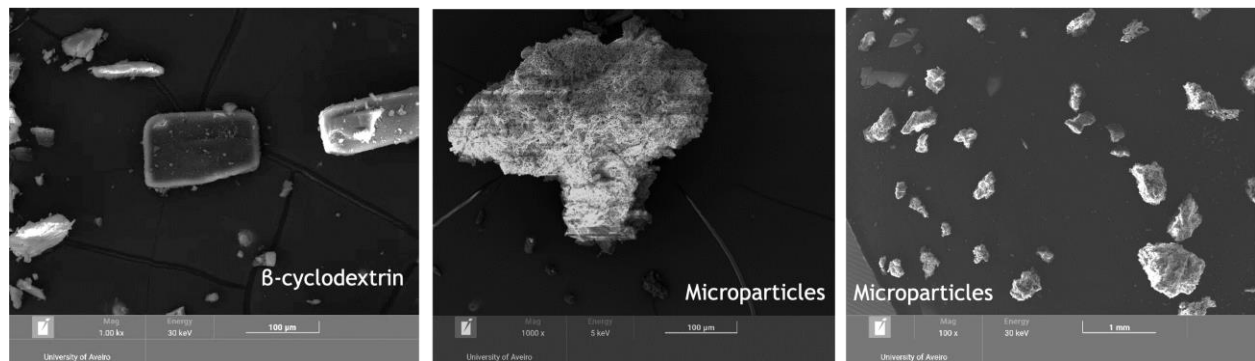


Figure 4.4- Results of the β -CD powder (coating agent) and OP extract microparticles analyzed by scanning electron microscopy (SEM).

The results reveal that the microparticles present an irregular shape, with some similarities with the β -CD powder morphology, in accordance with findings by several other authors for microparticles produced using β -CD powder as the coating agent (Ahmadi et al., 2023; Dehghani et al., 2020; Ganguly et al., 2022). Results also demonstrated that microparticles are porous, which may indicate that one of the phenomena that allows the release of the OP extract, from inside the microparticles, is by passing through the pores.

4.2.2 Thermal Stability of Microparticles

Microparticles were developed in this project to be incorporated in sunscreen formulations. Sunscreens are usually used in sunny and hot days. Hence, it is necessary to guarantee that these microparticles are thermal stable at high temperatures to ensure they can be incorporated in sunscreens. In order to evaluate the thermal stability of the microparticles, two analyses were performed: thermogravimetric analysis (TGA) and differential scanning calorimetry (DSC). TGA and DSC have been considered effective tests to study alterations in physical and chemical properties of the material caused by temperature (Abbastabar et al., 2020).

TGA analysis was performed in both β -CD powder and microparticles containing the OP extract; the results of the analysis are presented in Figure 4.5.

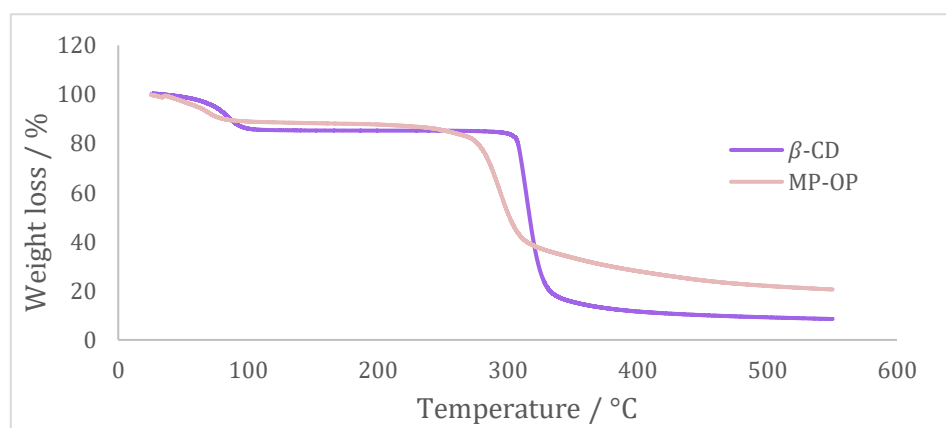


Figure 4.5- TGA analysis of the OP extract microparticles and β -CD powder.
 β -CD – β -cyclodextrin; MP-OP – microencapsulated OP extract.

TGA analysis reveals two thermal events of mass loss. The first event in the range of 20 and 105 °C, is due to the evaporation of adsorbed water on the microparticles surface. The second event from 250 to 400 °C corresponds to the decompositions and depolymerization of the microparticles constituents. MP-OP exhibits a lower degree of weight loss at 100 °C, suggesting that it possesses a lower moisture content in comparison to β -CD.

Moreover, the findings indicate that MP-OP demonstrates higher thermal stability compared to β -CD, with degradation occurring at approximately 250 °C. Consequently, caution should be exercised when using microparticles in applications or processes that involve temperatures exceeding this threshold.

Differential scanning calorimetry (DSC) analysis results are illustrated in Figure 4.6.

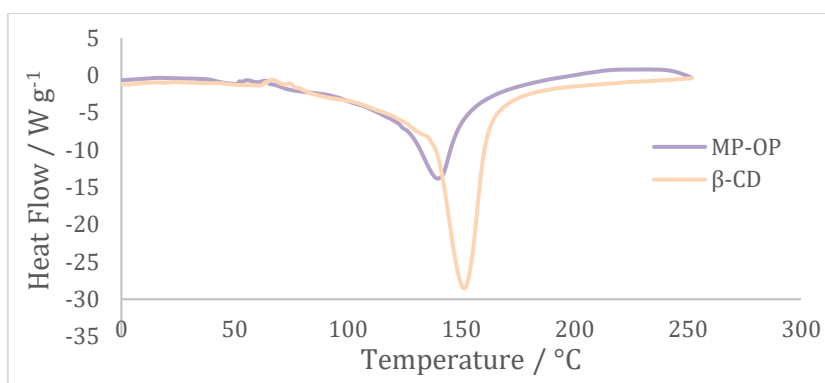


Figure 4.6- DSC analysis of both microparticles containing the OP extract (MP-OP) and β -CD powder.
 β -CD – β -cyclodextrin; MP-OP – microencapsulated OP extract.

Differential scanning calorimetry (DSC) is a widely employed and routine method for confirming the formation of complexes in the solid state (Ponce Cevallos et al., 2010). MP-OP and β -CD exhibited a broad endothermic event with a peak observed at approximately 140 °C for MP-OP and around 151 °C for β -CD. The endothermic peak of MP-OP shifted left by a couple of degrees in comparison of β -CD, presumably due to the interaction of OP extract with β -CD molecules.

The shift or disappearance of the endothermic melting peak observed on a DSC spectrum for a guest molecule is typically indicative of successful encapsulation or combination (Jiang et al., 2021). Therefore, the obtained results provide evidence of successful encapsulation of onion peel extract within β -cyclodextrin.

4.2.3 Photoprotection capacity

In order to evaluate the performance as a photoprotection agent of the microencapsulated extract and compare it with the performance of non-encapsulated the OP extract, the SPF value was calculated. The results are present in Table 4.7 and the absorbance spectra is illustrated in Figure 4.7.

Table 4.7 - SPF values for, non-encapsulated (OP) and microencapsulated (MP-OPE), OP extract solutions.

Sample concentration / mg mL ⁻¹	SPF
OP extract (0.095)	60.24
MP-OP (0.095)	36.11

SPF– Sun protection factor; OP – Onion Peel; MP-OP – Microencapsulated onion peel extract.

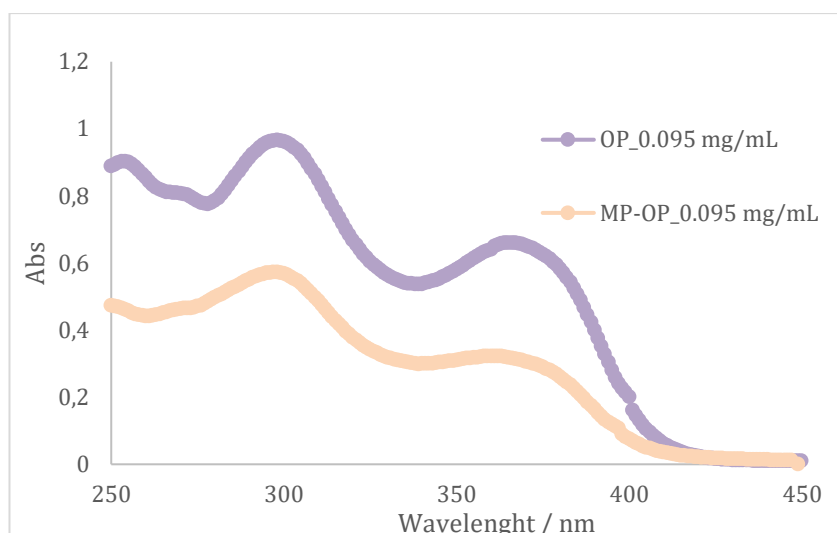


Figure 4.7- Absorbance spectra of, microencapsulated (MP-OP) and non-encapsulated (OP), OP extract solutions. OP – Onion Peel extract; MP-OP – microencapsulated OP extract.

Microparticles are characterized by having a controlled release of the trapped compound, in this case, there is a controlled release of the OP extract. It is expected that, for solutions of the same concentration, the solution of the microencapsulation extract presents a smaller amount of free extract, as a significant amount should still be entrapped at the time of the analysis. A smaller amount of free extract should lead to inferior absorbances as well as a smaller protection factor, which is demonstrated by the results presented in Table 4.7 and Figure 4.7.

4.3 Sunscreen Formulations

The formulations developed in the scope of this project were based on the formulations presented by Ferreira and Santos (2022). The formulations produced contain only the necessary ingredients to form a cream in order to allow the interpretation of the effect of the OP extract as an UV filter. The quantities of all the ingredients are within the limits of the legislation and in accordance with the literature values.

As previously described in Section 3.5, 5 oil-in-water formulations were produced. Synthetic UV filter oxybenzone was added at a concentration of 5% to formulation positive control (PC). This ingredient was chosen since it is one of the most widely used synthetic UV filters in commercial sunscreens (Geoffrey et al., 2019). To study the effect of OP extract in the formulations and compare its photo protectant performance to the performance of the synthetic UV filter, a formulation containing 5% of OP extract (OPE), non-encapsulated, was produced. In order to evaluate microencapsulation as a protection technique, a formulation containing 5% of microencapsulated OP extract (MP-OPE) was also developed. Finally, a formulation (MIX) containing a mixture of 1.6% of each of the three additives (oxybenzone, non-encapsulated and microencapsulated OP extract) was produced to perform a more thorough evaluation and comparison. Finally, as a negative control (NC), a formulation without additives was made. All formulations developed are represented in Figure 4.8.



Figure 4.8- Sunscreen formulations produced for this project.

NC – Negative control formulation; PC- Positive control formulation; OPE- Onion peel extract formulation; MP-OPE-Microencapsulated onion peel extract formulation; MIX-Mixture formulation.

4.3.1 Stability tests

After producing the sunscreens, the stability of the formulations was evaluated over a 5-week time span.

All formulations maintained organoleptic qualities, such a colour and smell, unaltered over the course of the study. NC and PC formulations had a beige colour, while the other formulations presented a brownish colour due to the presence of the OP extract. MP-OPE and MIX formulations were darker than the OPE formulation since it was necessary to use a bigger amount of microparticles (also brownish) to achieve the same percentage of OP extract, used in OPE formulation. In terms of aroma, NC and PC were odourless while the rest presented a light onion aroma. All creams demonstrated no changes in smell over the time of the study. Regarding their appearance/consistency, MP-OPE and MIX formulations had small visible particles while the remaining formulations were completely homogenous.

To evaluate formulations' physical stability, a centrifuge assay was performed, which results are illustrated in Figure 4.9. The centrifuge test revealed that none of the formulations presented phase separation, confirming the stability of the emulsions. Nevertheless, the undissolved granules were deposited at the bottom of the MIX formulation falcon.

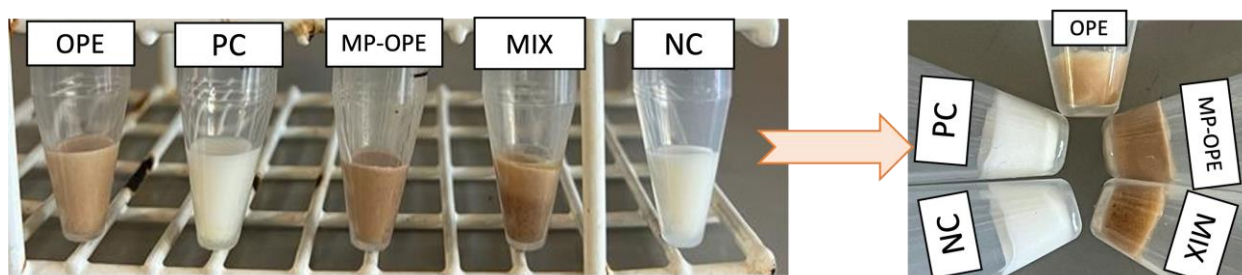


Figure 4.9 - Results of the centrifuge test for the week of production of the formulations and 4 weeks after production.

NC – Negative control formulation; PC- Positive control formulation; OPE- Onion peel extract formulation; MP-OPE-Microencapsulated onion peel extract formulation; MIX-Mixture formulation.

The formulations were also subjected to a thermal stability test to simulate the long-term shelf life. The results revealed no changes in colour, texture, smell or mass which indicates the emulsions present high long-term stability and maintain their properties regardless of temperature changes.

Skin pH is within the range of 4 to 6, and any cosmetic formulation produced with the intend of topical application should present values of pH within this range. The results of the pH value for each formulation at 3 different times of analysis are presented in Figure 4.10.

Results reveal that the pH of all formulation slightly decreases overtime. Nevertheless, all formulations' pH values remain within the ideal range for topical application. Furthermore, it is demonstrated that the presence of OP extract in the formulations acidifies them.

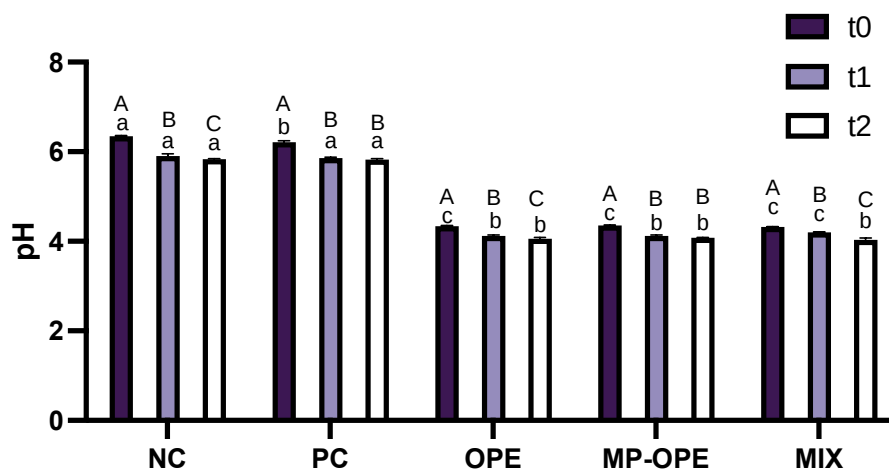


Figure 4.10 - pH values for the sunscreen formulations at the 3 analysis times.

NC – Negative control formulation; PC- Positive control formulation; OPE- Onion peel extract formulation; MP-OPE-Microencapsulated onion peel extract formulation; MIX-Mixture formulation. Different lowercase letters (a–d) in the same column represent statistically different values ($p < 0.05$) between the formulations for each analysis time. Different capital letters (A–B) in the same line, represent statistically different values ($p < 0.05$) between the two analysis times for each formulation. t0 – 2 days after production; t1 – two weeks after production; t2 – four weeks after production.

4.3.2 Antioxidant capacity

Antioxidants react with free radicals, neutralizing these and preventing the oxidation of the formulations (Hoang et al., 2021). Synthetic antioxidants are present in cosmetic formulations in low concentrations, for example butylated hydroxytoluene (BHT) is used in a concentration of 0.50% (Parliament, 2009). To study OP extract as an antioxidant, it would be necessary to produce formulation with 0.50% of OP extract and compare it with a positive control formulation, containing a synthetic antioxidant. However, the scope of this project was to evaluate the behavior of OP extract as an alternative UV filter and not as an antioxidant, and so, no formulation containing only 0.50% of OP extract was produced. Nevertheless, the antioxidant capacity of the formulations was evaluated by the DPPH assay, in order to elucidate us about the behaviour of phenolic extract in formulations. The results are illustrated in Figure 4.11.

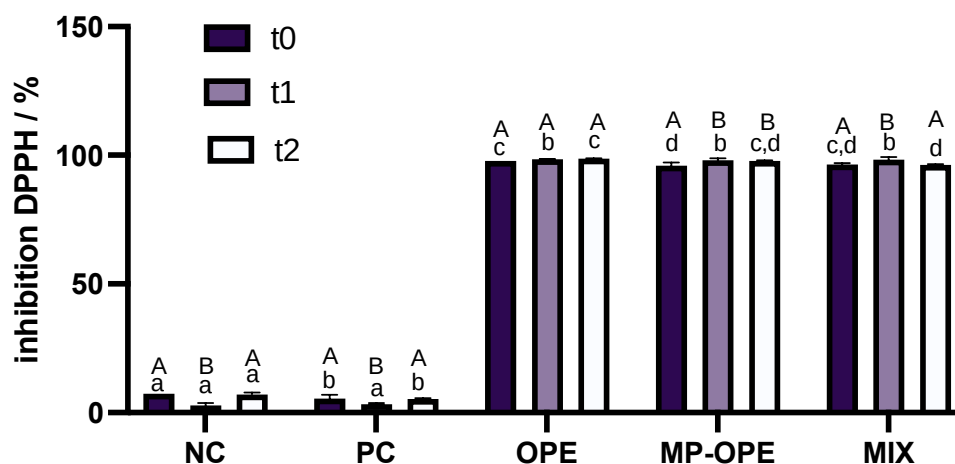


Figure 4.11- % of DPPH inhibition for the five formulations at the 3 analysis times.

NC – Negative control formulation; PC- Positive control formulation; OPE- Onion peel extract formulation; MP-OPE-Microencapsulated onion peel extract formulation; MIX-Mixture formulation. Different lowercase letters (a–d) in the same column represent statistically different values ($p < 0.05$) between the formulations for each analysis time. Different capital letters (A–B) in the same line, represent statistically different values ($p < 0.05$) between the two analysis times for each formulation. t0 – week of production; t1 – two weeks after production; t2 – four weeks after production.

The results reveal that neither NC or PC present antioxidant capacity, while the formulations containing the phenolic extract are able to inhibit almost all DPPH radical, presenting very strong antioxidant capacity. These observations are in accordance with the anticipated results, since NC and PC contain no antioxidant in their composition, and hence, no oxidation was prevented. Phenolic compounds are known for their antioxidant capacity and formulations containing the extract were expected to be able to postpone and reduce oxidation, which was demonstrated in the results.

Synthetic antioxidants' concentration on cosmetic formulations are limited by legislation. On the other hand, agricultural by-products extracts are constituted by several other bioactive compounds such as vitamins, which allows the use of these in higher concentrations increasing the antioxidant power of the formulations.

4.3.3 Photo-protection capacity

4.3.3.1 Sun Protection Factor

To evaluate the photo-protection capacity of the formulations, it was calculated the sun protection factor (SPF) of the formulations for 3 different times, and also, the variation of the protection factor between each time of analysis. The results are presented in Table 4.8 and Figure 4.12. The absorbance spectra of these formulations, used to calculate the SPF value, is presented in Figure F.0.1 in Appendix F.

Table 4.8 - Sun Protection Factor (SPF) values for the different formulations at the 3 analysis times.

Sample	SPF t0	SPF t1	SPF t2
OPE	4.68 ± 0.07 ^{a,A}	2.05 ± 0.27 ^{a,B}	1.38 ± 0.35 ^{a,C}
MP-OPE	18.22 ± 0.50 ^{b,A}	10.96 ± 0.00 ^{b,B}	6.27 ± 0.01 ^{b,C}
MIX	12.93 ± 0.14 ^{c,A}	11.21 ± 0.40 ^{b,B}	11.00 ± 0.25 ^{c,B}
NC	0.83 ± 0.13 ^{d,A}	0.24 ± 0.15 ^{c,A,B}	0.00 ± 0.00 ^{d,B}
PC	22.82 ± 0.17 ^{e,A}	18.99 ± 0.10 ^{d,B}	17.79 ± 0.02 ^{e,C}

NC – Negative control formulation; PC- Positive control formulation; OPE- Onion peel extract formulation; MP-OPE-Microencapsulated onion peel extract formulation; MIX-Mixture formulation. Different lowercase letters (a–e) in the same column represent statistically different values ($p < 0.05$) between the formulations for each analysis time. Different capital letters (A–C) in the same line, represent statistically different values ($p < 0.05$) between the two analysis times for each formulation. t0 – week of production; t1 – two weeks after production; t2 – four weeks after production.

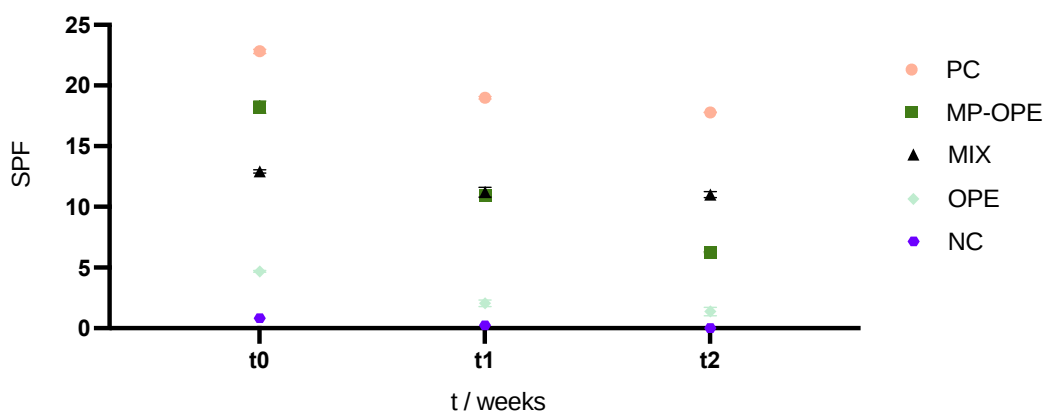


Figure 4.12 - SPF value variation between analysis time for the sunscreen formulations.

NC – Negative control formulation; PC- Positive control formulation; OPE- Onion peel extract formulation; MP-OPE-Microencapsulated onion peel extract formulation; MIX-Mixture formulation. t0 – week of production; t1 – two weeks after production; t2 – four weeks after production.

The results reveal that NC presents no protection factor against UV radiation, which was expected since no additive/UV filter/ extract was added to this formulation. PC formulation presented the highest value of SPF.

In terms of the formulations containing OP extract, the analysis is more complex. In the week of production of the sunscreens, formulation containing microencapsulated extract (MP-OPE) presented a higher protection factor than both the OPE and MIX formulations. For the second analysis time, two weeks after production, and for the final analysis time, four weeks after production, MIX emulsion presents the highest SPF value. OPE formulation is always the formulation presenting the lowest protection factor, which may be justified by the fast degradation of the OP extract while not protected. Considering the variation of the protection factor, depicted in Figure 4.12, it is possible to further discuss the results presented above. The results reveal that, between t0 and t2, MP-OPE formulation presents a constant decrease in the SPF value, which may be justified by the controlled release of the OP extract, when microencapsulated. Extract release from the microparticles is expected to peak in the first few hours, followed by a constant release of the extract from that point on. Considering that the first analysis was performed two days after production of

the cream (t_0), the microparticles in the formulation should be releasing OP extract at a constant rate, leading to a proportional decrease in the SPF value with time. To confirm this theory, kinetic assays to study the controlled release must be performed in future work. On the other hand, the OPE formulation contains no encapsulated extract which leads to a greater decline in the value of the SPF between the first two analysis. At t_1 (two weeks after production), the majority of the OP extract had already been degraded. The existence of a lesser amount of extract in the formulation between t_1 and t_2 (four weeks after production), lead to a lesser abrupt decrease in the protection factor value.

These results reveal that microencapsulation protects the extract from degradation, and, when incorporated in cosmetic formulations, enables sunscreens to maintain their photo protection for longer periods of time, increasing sunscreen shelf life. MIX and PC formulation demonstrated the best results, presenting almost no decline in the SPF value. The results suggest that the OP extract, either free or microencapsulated, presents itself as a prospect natural UV filter to substitute synthetic UV filters or combined with these to decrease the use and concentration of the latter.

4.3.3.2 Photoprotection evaluation – skin reaction

The photoprotection capacity of the different formulations was evaluated by studying a volunteer's skin reactivity to the formulations after one hour of sunlight exposure. The results are depicted in Figure 4.13.

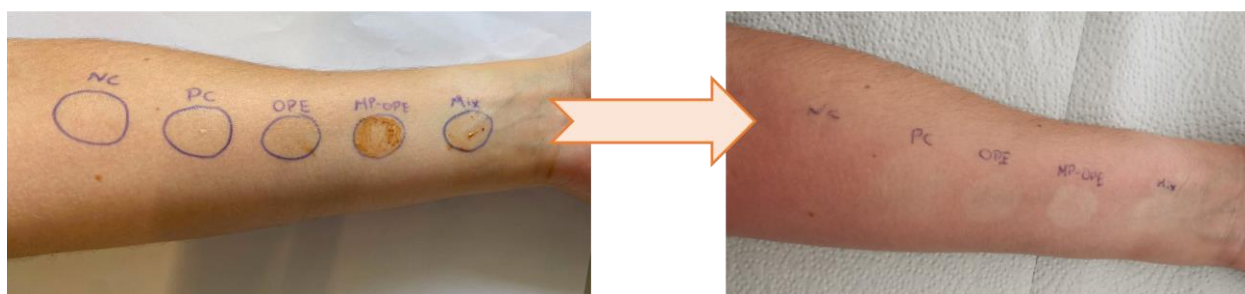


Figure 4.13 - Volunteer's skin reaction before and after 1 hr of sunlight exposure. The first photograph was taken right before sun exposure, and the second was taken 3 hours after the skin being exposed to 1 hr of sunlight. Skin reaction test was performed 4 weeks after production of the sunscreen formulations.

NC – Negative control formulation; PC- Positive control formulation; OPE- Onion peel extract formulation; MP-OPE-Microencapsulated onion peel extract formulation; MIX-Mixture formulation.

As expected, the skin area to which NC formulation was applied displayed the most irritation as this formulation had no additive to provide protection against UV radiation. All the other formulations demonstrated protection against UV radiation, which is in concordance with the results obtained by the SPF value test. Visually, it appears that formulations containing OP extract provide higher protection, with MP-OPE and MIX formulation presenting the best results, with the skin area where they were applied appearing less tanned and irritated than the rest. Figure 4.13 shows that OPE formulation presents higher protection than the PC but protect less against UV radiation than MP-OPE and MIX formulations. The results reveal that by microencapsulating the extracts, protection of the OP extract is successfully accomplished, preserving the photo-protection capacity of the extract and enhancing its protection against UV radiation.

The results of this test appear to contrast with the obtained SPF values. However, SPF only evaluates photoprotection in UVB region. OP extract is a mixture of compounds that might absorb UV radiation in other regions of UV radiation, providing higher protection than what the SPF value reveal.

Even though these are preliminary studies, they suggest that OP extract, either non-encapsulated or microencapsulated, might be used as a source of photoprotection for more sustainable and environmentally friend sunscreens.

5 Conclusion

The primary purpose of this dissertation was to investigate the possibility of using onion peel extracts as a natural UV filter to develop a value-added sunscreen, while decreasing the concentration of synthetic UV filters in the formulations or even fully replacing these with natural UV filters.

The onion peel (OP) extract was obtained by solid-liquid extraction with a Soxhlet apparatus. Ethanol was used as solvent and the resulting extraction yield was $(15.72 \pm 1.79)\%$, which is in accordance with the values found in the literature. The main phenolic compounds identified and quantified by HPLC-DAD were quercetin, in a concentration of $(4.48 \pm 0.01) \text{ mg g}^{-1} \text{ dried extract}$, and $(0.51 \pm 0.02) \text{ mg g}^{-1} \text{ dried extract}$ of resveratrol. The results are found to be inferior to values found in the literature. The studies found in the literature analyse different variety of onion peels. The composition of the peels depends on the variety of the onion that is evaluated, which might justify the difference in terms of concentration of the main phenolic compounds. The antioxidant capacity of the OP extract was evaluated by three assays: DPPH, ABTS and Folin-Ciocalteu. Folin-Ciocalteu assay was used to calculate the total phenolic content of the phenolic extract, and a value of $(585.23 \pm 10.43) \text{ mg}_{\text{GAE}} \text{ g}^{-1} \text{ extract}$ was obtained. The ABTS assay revealed an antioxidant capacity of $(381.45 \pm 8.05) \text{ mg}_{\text{TEAC}} \text{ g}^{-1}$. Finally, the OP extract analysed by the DPPH assay demonstrated an antioxidant capacity of $(199.71 \pm 1.16) \text{ mg}_{\text{TE}} \text{ g}^{-1}$ and an IC_{50} of $(31.01 \pm 1.98) \text{ mg L}^{-1}$, suggesting that OP extract is a very strong antioxidant. The results are within the range of values found in the literature for these assays. A high antioxidant capacity is very important in cosmetic formulations since it protects them from lipid oxidation that originates compounds with an unpleasant aroma and degrades the formulation. The photoprotection capacity was evaluated by calculating the sun protection factor (SPF). SPF studies revealed that solutions containing OP extract provide significantly less photoprotection ($\text{SPF} = 6.05$) than solutions containing the commercial synthetic UV filter ($\text{SPF} = 38.84$), for the same mass concentration. However, the phenolic extract is a mixture of compounds as opposed to a single compound as the oxybenzone, suggesting that it should have been used in a higher concentration. The results for higher concentrations of OP extract demonstrated that by increasing the concentration of OP extract solutions, it was achieved a higher value of SPF, 60.24, than that obtain for the synthetic UV filter, implying that OP extract has high potential as a natural UV filter.

Additionally, OP extract was microencapsulated by molecular inclusion, using β -cyclodextrin as coating agent, to protect the phenolic extract against oxidation, preserve its bioactive agents for longer periods. The microencapsulation process revealed an encapsulation efficiency of $(91.80 \pm 0.03)\%$, a mean diameter of $(30.84 \pm 22.14) \mu\text{m}$, and a size distribution with a polydispersity of 2.09. Regarding shape, the microparticles obtained appeared in irregular shape, as expected for microencapsulation techniques using β -cyclodextrin. SEM analysis also shown that microparticles are porous, which may constitute a strategy to the release of the OP extract from inside the particles. Microparticles containing the phenolic extract were also evaluated for their antioxidant power and their photoprotection capacity, using the DPPH assay and calculating the SPF value, respectively. In terms of their antioxidant capacity, the results obtained for the DPPH assay

were 303.76 mg_{TE} g⁻¹_{extract} and an IC₅₀ of 23.66 mg L⁻¹. Previous studies had demonstrated that microencapsulation of quercetin and resveratrol (the main compounds of OP extract) enhanced their antioxidant capacity, which is in accordance with the obtained results. The calculation of the SPF value shown the microparticles presented an inferior protection factor in comparison of the non-encapsulated extract. The difference may be caused by the controlled release that occurs in microparticles, which leads to an inferior concentration of free OP extract in the solution. Finally, OP extract microparticles were evaluated for their thermal stability, using thermogravimetric analysis (TGA) and differential scanning calorimetry (DSC). Microparticles exhibit a lower degree of weight loss at 100°C than β-CD, suggesting that they possess a lower moisture content in comparison to β-CD. Moreover, the findings indicate that microparticles demonstrate higher thermal stability compared to β-CD, with degradation occurring at approximately 250°C. The obtained results with DSC analysis provide evidence of successful encapsulation of onion peel extract within β-CD.

Additionally, the extracts (non-encapsulated and microencapsulated) were incorporated into oil-in-water emulsions to evaluate their antioxidant capacity, stability, and sun protection factor (SPF). The pH of the formulations containing the extracts revealed values between 4.00 and 6.00, values within the normal range for topical application. All the formulations were stable after performing the thermal and physical stability tests. The formulations containing OP extract demonstrated great antioxidant capacity (around 98% of inhibition of DPPH radical), in opposition to the negative (NC) and positive (PC) control (containing synthetic UV filter) that showed no antioxidant capacity. Regarding their photoprotection potential, two factors were evaluated: Sun Protection Factor and skin's reactivity. In the evaluation of the SPF value, the results reveal that NC presents no protection factor against UV radiation, which was expected since no additive/UV filter was added to this formulation. At the week of production, for the formulations containing OP extract, the microencapsulated extract formulation presented a higher value of SPF than the non-encapsulated counterpart and the formulation containing a mixture of the three additives (MIX) (non-encapsulated, microencapsulated and synthetic UV filter). The MIX formulation was the emulsion containing OP extract that better maintained the SPF value throughout the assay, remaining mostly constant over 5 weeks. PC presented the highest SPF value and was able to maintain it approximately constant along the time. The skin's reactivity test revealed that the formulations containing OP extract, either free, encapsulated or a mixture, protected the skin from sunlight exposure.

Overall, the results allow to infer that OP extract, either free or encapsulated, presents itself as a prospective natural UV filter to substitute synthetic UV filters, or combined with these, to decrease the utilization and concentration of the latter. Microencapsulating the OP extract ensured the protection of the photoprotector capacity of the extract for longer periods of time, increasing the shelf life of the sunscreen. Nevertheless, given the preliminary phase of the tests performed, further analysis of the non-encapsulated, microencapsulated OP extract and the formulations must be carried out, such as rheology tests, controlled release studies and lipid oxidation assays.

6 Assessment of the work done

6.1 Objectives Achieved

The work realized for this project met the proposed objectives. Onion peel (OP) extract was obtained and characterized, confirming a high antioxidant and photoprotection capacity. The extract was microencapsulated, and the microparticles were characterized regarding their physical and chemical properties. Sunscreen formulations containing non-encapsulated and microencapsulated OP extracts were produced to evaluate their potential as a natural UV filter. However, even though the results were promising, only preliminary tests were done due to time limitations, and thus, further studies must be performed.

6.2 Future Work

It would be valuable to assess the rheologic properties of the OP extract, specifically by performing viscosity tests. Additionally, it would be recommended to perform acute, subacute and sub chronic toxicity evaluations, in order to identify possible adverse side effects that may emerge with the use of these extracts, as well as to determine their maximum dosage level. As an agricultural by-product, onion peels come from agricultural cultivations that might use pesticides. Hence, identification and quantification of potential pesticides must be performed to guarantee the safety of the extracts.

Furthermore, to better evaluate the microparticles behavior, kinetic studies in water (solvent used for the microencapsulation process) and in the sunscreen formulations, must be carried out.

Moreover, other stability tests on the sunscreen formulations are recommended, such as determination of formulations spreadability, as well as spreadability variation along time. Phenolic compounds are highly sensitive and oxidize easily and thus, it would be interesting to study the lipid oxidation of the formulation along time, to evaluate whether the obtained microparticles are successfully protecting the extract. Skin compatibility must also be studied.

6.3 Final Assessment

Cosmetic industry always fascinated me because it produces products that make people feel confident and good about themselves. However, this industry uses reagents and synthetic compounds that are harmful and have nefarious environmental impact. This project allowed me to develop a cosmetic product, that not only protects the skin from harmful effects caused by UV radiation, but also, allows people to safely achieve the skin tone that provides that extra confidence, while contributing to a circular economy by adding a new step in the life of agricultural by-products. This work made me grow personally and professionally, by developing new soft and hard skills, as well as utilizing many of the acquired during the last 5 years.

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Annex A - Sun Protection Factor

A.1 Sun Protection Factor Calculation

Sun protection values are calculated through Equation 3.6. Table A.1.0.1 presents the values of the normalized product function needed for the calculations, obtained from Mukund Manikrao & Sharada (2016).

Table A.1.0.1 – Normalized Product function values for each wavelength.

Wavelength / nm	$EE(\lambda) \times I(\lambda)$
290	0.0150
295	0.0817
300	0.2874
305	0.3278
310	0.1864
315	0.0839
320	0.0180
total	1

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Appendix A - Literature review

A.1 Microencapsulation techniques used for the encapsulation of several plant extracts

As described in Section 2.3, the microencapsulation of a wide range of phenolic compounds and plant extracts using various methods has garnered significant interest in recent years. To compile relevant studies, a comprehensive literature search was conducted from 2014 to 2023, utilizing reputable databases such as Scopus, PubMed, and Google Scholar. The search strategy involved incorporating keywords such as “extract”, “encapsulation”, and “phenolic compounds”, leading to the identification and inclusion of pertinent studies in Table A.0.1.

Table A.0.1- Microencapsulation techniques used for the encapsulation of different plant extracts.

Encapsulation method	Core material	Wall material	Results	Reference
Hydrogel	Onion peel extract	Soy protein isolate	High EE (97.34%) and increased stability and bio accessibility.	(Stoica et.al, 2022)
Freeze drying	Onion peel extract	B-LG hydrolysates	EE (61.07%) and increased antioxidant capacity	(Milea et al., 2021)
Freeze drying	Onion peel extract	Maltodextrin	High EE 89.15% and increased thermal stability	(Akdeniz et al., 2018)
Freeze drying	Onion peel extract	Maltodextrin	High EE 94.30% and higher concentration of polyphenols and antioxidant capacity than the non-encapsulated extract	(Elsebaie & Essa, 2018)
Liposomes	Hibiscus extract	Soybean lecithin	High EE (62.7%) and improved stability	(Gibis <i>et al.</i> , 2014)
Liposome	Elderberry extract	Soybean lecithin	Stable liposomes, with a uniform structure, small size (205 nm), but with a poorer value of EE (around 25%)	(Bryla <i>et al.</i> , 2015)
Molecular inclusion	Blackberry purees	B-cyclodextrin	Protected from thermal degradation and increased stability and bioaccessibility	(Fernandes <i>et al.</i> , 2018)

Gastroretentive systems	Haskap berries extract	Alginate-based in situ gelling system	Modulated of the release and absorption	(Celli <i>et al.</i> , 2016)
Gastroretentive systems	<i>Clitoria ternatea</i> petal flower extract	Alginate-based in situ gelling system	Reduced degradation and increased biological activity after gastrointestinal digestion; EE 84.87%	(Pasukamonset <i>et al.</i> , 2016)
Double emulsion	Anthocyanins commercial extract	Grape seed oil and guar gum	High EE (90.6%) and kinetic stability and protected against degradation	(Paula <i>et al.</i> , 2018)
Double emulsion and biopolymer particles	Black raspberry ethanolic extract	Soybean oil / gelatin and gum arabic	Advantage in color conservation and improved thermal stability; EE 81.12%	(Shaddel <i>et al.</i> , 2018)
Emulsion	Bilberry extract	Bilberry seed oil / whey protein isolate	Stable emulsion structure and protected from degradation	(Syanberg <i>et al.</i> , 2019)
Emulsion	Black carrot concentrates	Sunflower oil / whey protein isolate	Protected the color by increasing its stability	(Bilek <i>et al.</i> , 2017)
Biopolymer particles	Purple corn and blueberry extracts	Alginate and pectin	Reduced the photodegradation rate and increased the shelf life; EE 65 and 116% respectively	(Guo <i>et al.</i> , 2018)
Biopolymer particles	Blueberry extracts	Chitosan	Improved the EE (94.02%) and stability	(Wang <i>et al.</i> , 2017)
Spray-drying	Jabuticaba peel extract	Chitosan	High ee (89.74%) and improved thermal stability;	(Cabral <i>et al.</i> , 2018)
Spray-drying	Maqui juice	Inulin or sodium alginate	Improved the stability and increased the bioaccessibility; EE 68.6 and 47.3% respectively	(Fredes <i>et al.</i> , 2018)
Spray-drying and freeze-drying	Bordo grape skin extract	Gum arabic, partially hydrolyzed guar gum, and polydextrose	Degradation in the freeze-dried powder is more sensitive to temperature than in the spray-dried powder	(Kuck <i>et al.</i> , 2017)
Freeze-drying	Jabuticaba pomace extract	Maltodextrin, pectin, and soy protein isolate	Protection to monomeric anthocyanin higher than 76% and improved the stability	(Souza <i>et al.</i> , 2017)
Freeze-drying	Blackberry extract	Maltodextrin	High anthocyanin retention and EE (76%)	(Yamashita <i>et al.</i> , 2017)

Freeze-drying	Sour cherries extract	B-lactoglobulin	Protect the anthocyanins from the gastric digestion, facilitating their release into the intestine; EE 64.69%	(Oancea <i>et al.</i> , 2017)
Electrospraying	Black carrot extract	Chitosan and gelatin	Faster and greater released in the food simulant acetic acid medium; EE 76.9%	(Atay <i>et al.</i> , 2018)
Electrospinning	Sour cherry concentrate	Gelatin or gelatin-lactalbumin	Increased the stability and the bioaccessibility; EE 79.2 and 70.2% respectively	(Isik <i>et al.</i> , 2018)
Sas	Blackberry extract	Polyvinylpyrrolidone	Dry product richer in anthocyanins and with enhanced purity; improved thermal stability	(Machado <i>et al.</i> , 2018)
Pgss	Elderberry juice concentrate	Palm fat	Homogeneously colored free-flowing fine powder rich in anthocyanins and color stability	(Bánvölgyi <i>et al.</i> , 2016)
Spray-drying and molecular inclusion	Saffron petals extract	B-glucan or β -cyclodextrin	Increased the bioaccessibility in intestinal section; EE 45 and 63.25% respectively	(Ahmad <i>et al.</i> , 2018)
Spray-drying and biopolymer particles	Red grape juice	Soy protein or whey protein / maltodextrin	Improved thermal stability and increased the shelf life	(Moser <i>et al.</i> , 2017)
Supercritical carbon dioxide and liposome	Commercial anthocyanin from bilberry	Soy lecithin	Small size (160 nm), high stability and EE (52.2%)	(Zhao <i>et al.</i> , 2017)
Double emulsion and spray-drying	Anthocyanin powder from the black carrot	Glycerol mono-oleate and soy lecithin / maltodextrin and poloxamer 338	Protected against heat degradation from thermal processing; EE 57.2%	(Ravanfar <i>et al.</i> , 2018)
Double emulsion, complex coacervation and freeze-drying	Black raspberry extract	Gelatin and arabic gum	Improved thermostability, conservation of color, long residual action of anthocyanins after being encapsulated; EE 81.12% at	Shaddel <i>et al.</i> 2018b)
Biopolymer particles and freeze-drying	Elderberry extract	Whey protein and pectin	Higher stability when subjected to thermal treatment; EE 97.13%	(Stănciuc <i>et al.</i> , 2018)
Freeze-drying, complex coacervation and molecular inclusion	Blueberry extract	Maltodextrin, β -cyclodextrin, whey protein isolate and gum arabic	Protect anthocyanins against degradation during heating; EE 82%	(Tao <i>et al.</i> , 2017)

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Appendix B - Radical Scavenging Capacity

B.1 - DPPH Assay

As described in Section 3.3.2, the microplate method was followed to evaluate the antioxidant capacity of the OPE through the DPPH assay. Afterwards, the absorbance was read at a wavelength of 515 nm and the resulting microplate is presented in Figure B.0.1.

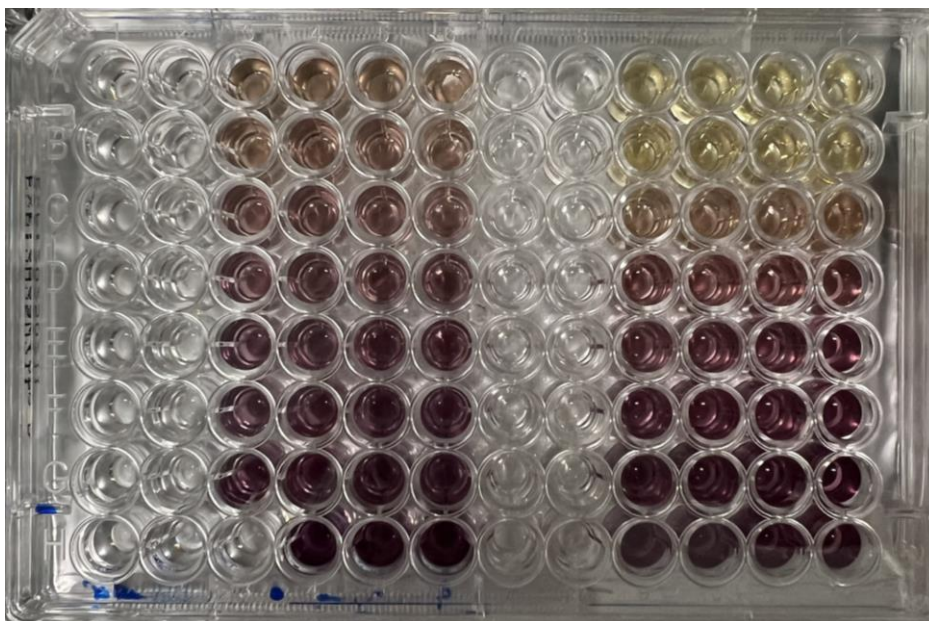


Figure B.0.1 - Resulting microplate after DPPH assay.

As the results were presented in Trolox equivalents antioxidant capacity, the Trolox calibration curve was prepared and is illustrated in Figure B.0.2 and presented in Equation (B.1.1), where C_{Trolox} (mg L^{-1}) is the Trolox concentration.

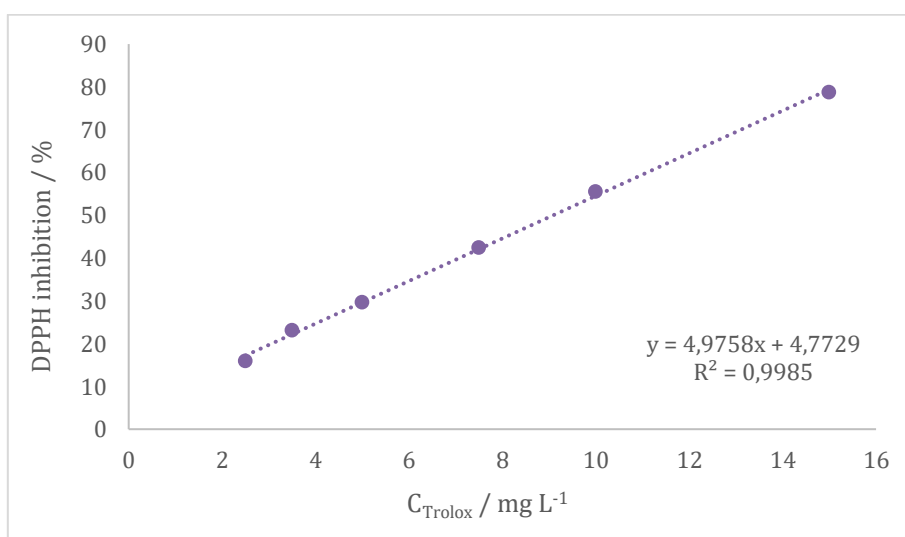


Figure B.0.2 - Trolox Calibration Curve.

$$\text{DPPH inhibition \%} = (4.98 \pm 0.19) C_{Trolox} + (4.77 \pm 1.41) \quad (\text{B.1.1})$$

The half maximal inhibitory concentration IC_{50} (mg L^{-1}), which represents the concentration of extract/Trolox needed to inhibit 50% of the DPPH present in solution. It was calculated following Equation (B.1.2), where *Intercept* is the intercept of the *DPPH inhibition %* curve and *Slope* represents the slope of the same curve.

$$IC_{50} = \frac{50 - \text{Intercept}}{\text{Slope}} \times 1000 \quad (\text{B.1.2})$$

Finally, the radical scavenging capacity of OPE, expressed in TE ($\text{mg}_{TE} \text{g}^{-1}_{\text{extract}}$), was obtained by following Equation (B.1.3).

$$TE = \frac{IC_{50}(\text{trolox})}{IC_{50}(\text{sample})} \times 1000 \quad (\text{B.1.3})$$

B.2 - ABTS Assay

To perform the ABTS method, it was applied the microplate method and the absorbance values were read at a wavelength of 734nm. The resultant microplate after incubation is shown in Figure B.0.3.

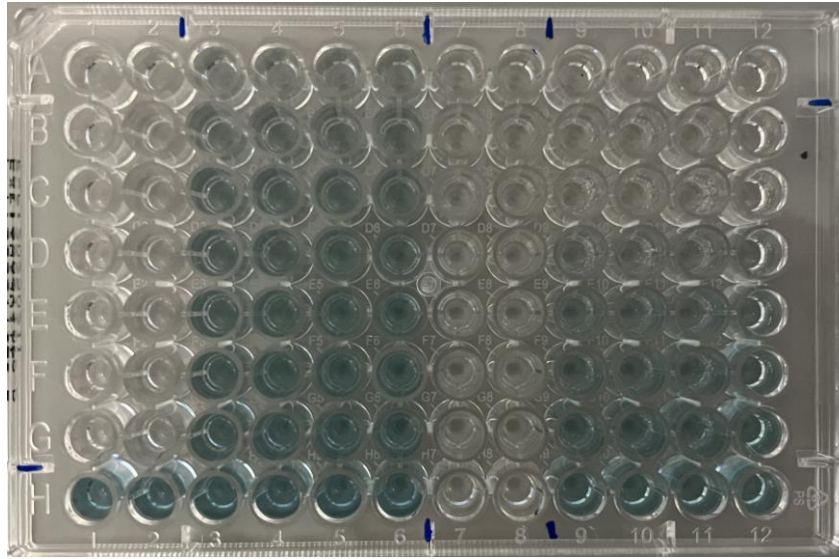


Figure B.0.3 - Resulting microplate of ABTS assay.

As the results were presented in Trolox equivalents antioxidant capacity, the Trolox calibration curve was prepared and is illustrated in Figure B.0.4 and presented in Equation (B.2.1), where C_{Trolox} (mg L^{-1}) is the Trolox concentration.

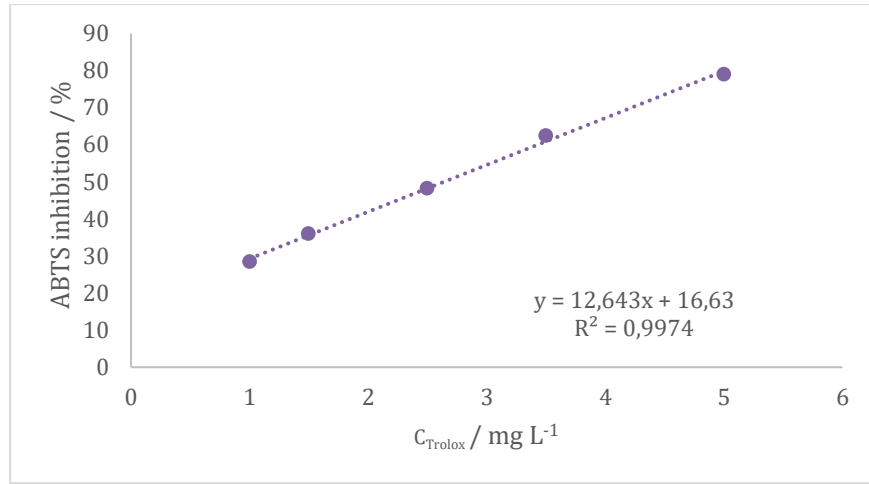


Figure B.0.4 - Trolox Calibration Curve.

$$ABTS \text{ inhibition } \% = (12.64 \pm 0.47) C_{Trolox} + (16.63 \pm 1.75) \quad (\text{B.2.1})$$

After calculating the % of ABTS inhibition, it was calculated the half maximal inhibitory concentration (IC_{50}) (mg L^{-1}) through Equation (B.2.2), where *Intercept* is the intercept of the *ABTS inhibition* % and *Slope* represents the slope of the same curve.

$$IC_{50} = \frac{50 - \text{Intercept}}{\text{Slope}} \times 1000 \quad (\text{B.2.2})$$

The radical scavenging capacity of OPE were expressed in *TEAC* ($\text{mg}_{TEAC} \text{ g}^{-1}_{\text{extract}}$) following Equation (B.2.3).

$$TEAC = \frac{IC_{50}(\text{trolox})}{IC_{50}(\text{sample})} \times 1000 \quad (\text{B.2.3})$$

Appendix C - Onion Peel Extract Characterization

C.1 - Extraction of Phenolic Contents

Figure C.0.1, illustrates onion peels after pre-treatment and before solid-liquid extraction, the extraction apparatus, the liquid OP extract, and the solvent of the extraction being evaporated in rotavapor.

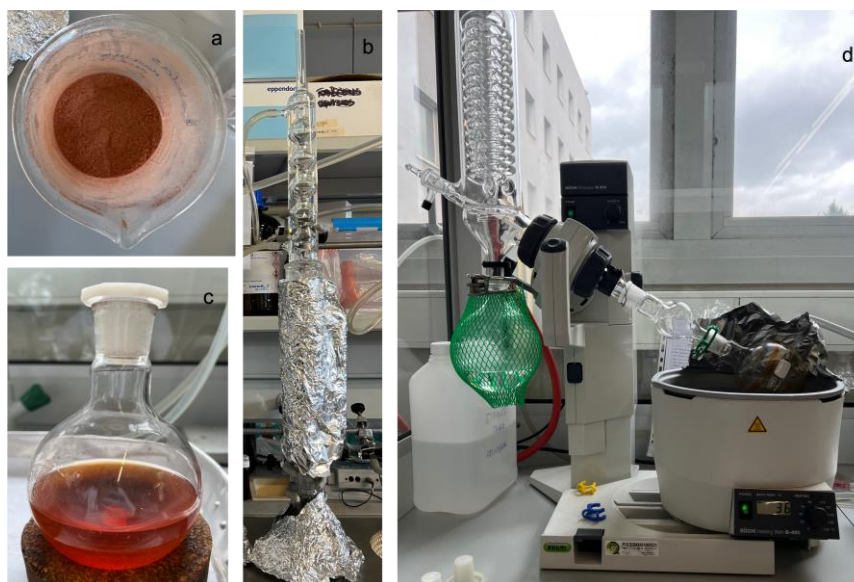


Figure C.0.1 - a - Onion Peels after pre-treatment; b - Solid-liquid extraction apparatus; c - Liquid onion peel extract; d - Solvent being evaporated in rotavapor.

C.2 - HPLC-DAD Chromatograms

Equations C.2.1 and C.2.2 exhibit the validated calibration curves obtained in the HPLC-DAD for quercetin and resveratrol, respectively, with R values of 0.9994 and 0.9999. These curves were validated by Ferreira et al. (2023), the research group that supervised this work. The curves were calculated in the same laboratory, HPLC equipment and conditions.

$$A = 73700 \times C_{\text{quercetin}} - 268000 \quad (\text{C.2.1})$$

$$A = 142000 \times C_{\text{resveratrol}} + 279000 \quad (\text{C.2.2})$$

Where $C_{\text{quercetin}}$ (mgL^{-1}) and $C_{\text{resveratrol}}$ (mgL^{-1}) represent the concentration of quercetin and resveratrol in the sample, respectively and A represents the area under the peak.

Figure C.0.2 and Figure C.0.3 illustrate the chromatograms obtained by HPLC-DAD for OP extract and quercetin standard + OP extract, in order to identify and quantify quercetin in the sample. Quercetin was identified at a retention time of 49.98 min and wavelength 365 nm.

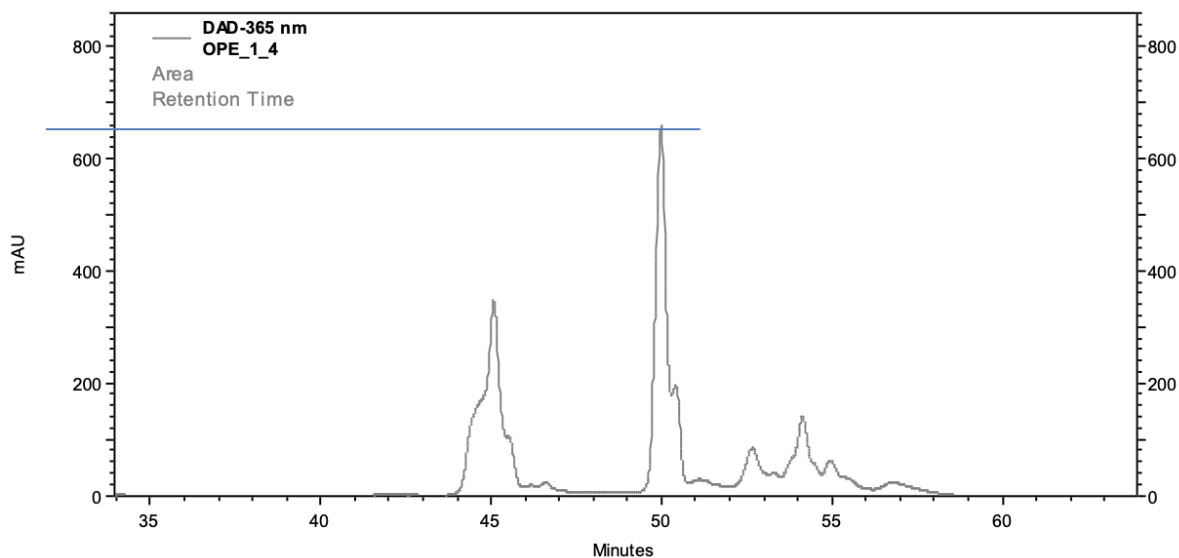


Figure C.0.2 - Zoomed in chromatogram of the OP sample at $\lambda = 365$ nm.

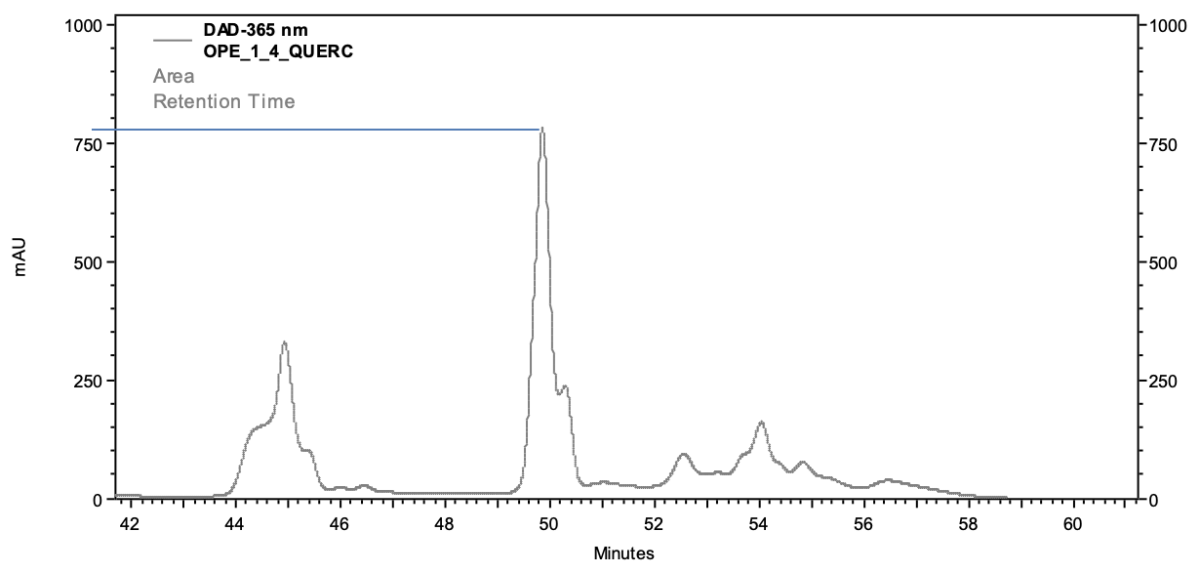


Figure C.0.3 - Zoomed in chromatogram of the quercetin standard + OP extract sample at $\lambda = 365$ nm.

Figure C.0.4 and Figure C.0.5 illustrate the chromatograms obtained by HPLC-DAD for OP extract and resveratrol standard + OP extract, in order to identify and quantify quercetin in the sample. Quercetin was identified at a retention time of 44.44 min and wavelength 305 nm.

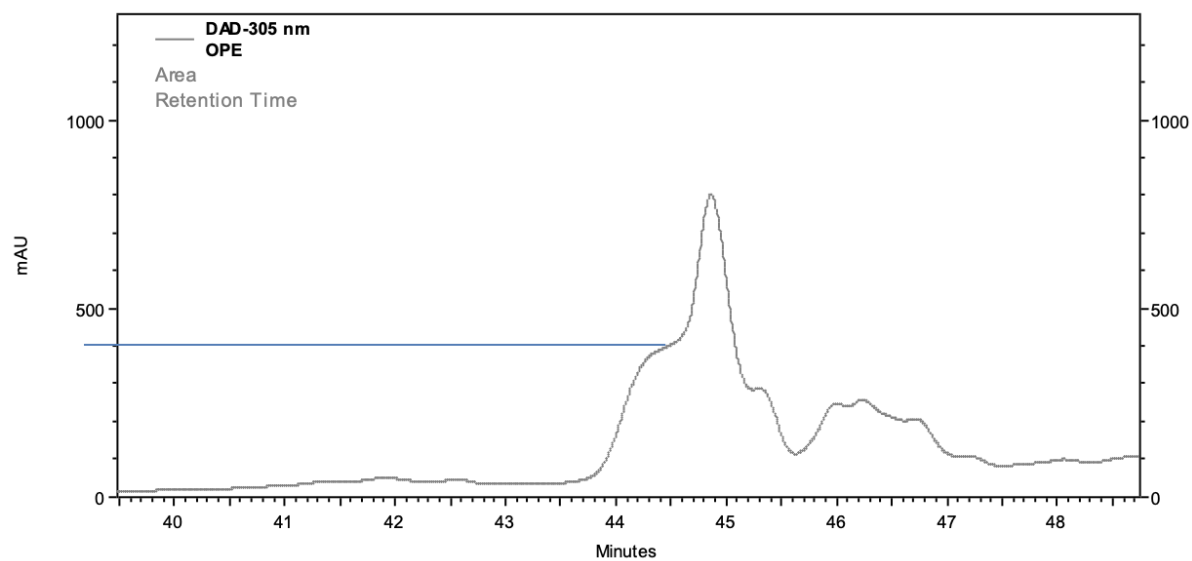


Figure C.0.4 - Zoomed in chromatogram of the OP extract sample at $\lambda = 305$ nm.

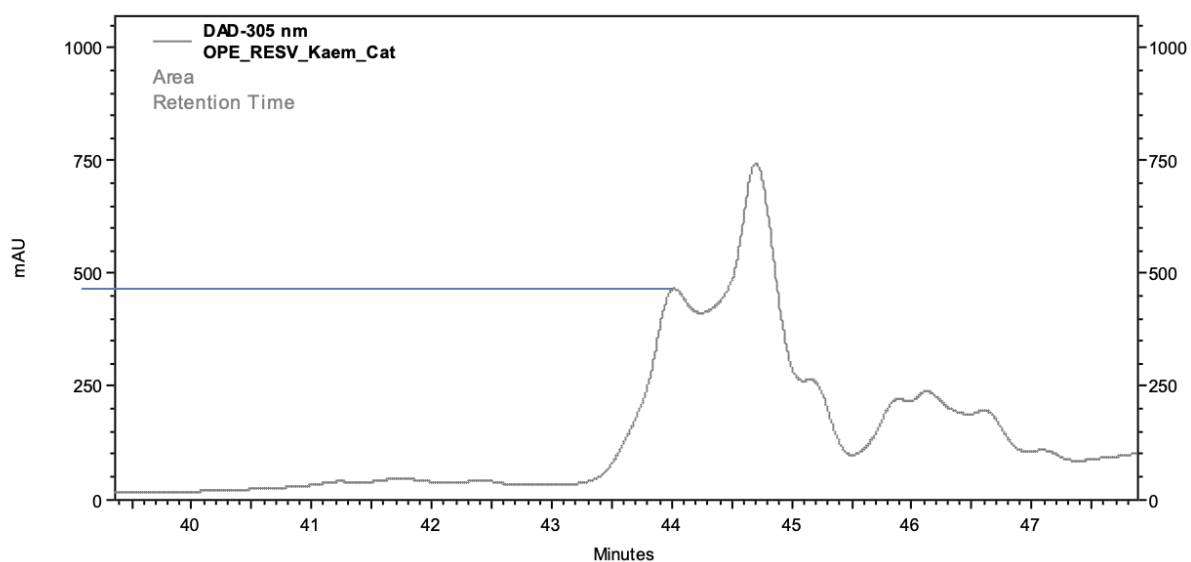


Figure C.0.5 - Zoomed in chromatogram of resveratrol standard + OP extract sample at $\lambda = 305$ nm

Appendix D - Encapsulation

D.1 - Microencapsulation Process

Microencapsulation process was performed as described in Section 3.5. Figure D.0.1 illustrates some steps in production of microparticles.

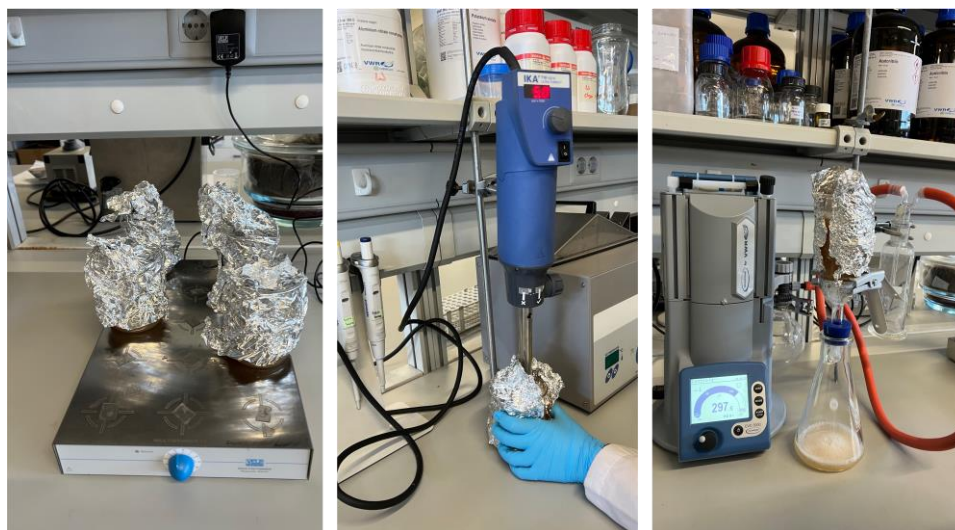


Figure D.0.1 – Microencapsulation steps: Magnetic stirring for 2 hr, homogenization by ultraturax and vacuum filtration, from left to right.

D.2 - Calibration Curve Validation

A validation method was performed on the calibration curve to ensure the suitability of this method to calculate the encapsulation efficiency.

The calibration curve was validated by quantification parameters (sensitivity, linearity, limit of detection and limit of quantification).

A linear curve is considered valid and suitable for use in quantification methods when:

- At least 5 standard solutions are used;
- Concentrations range should present a factor of at least 10;
- The correlation coefficient, R^2 , should be at least 0.995;
- The relative standard deviation, S_a/a , must be inferior to 5%;
- $b - t \times S_b < 0 < b + t \times S_b$.

Where a is the slope, b the intercept, and their standard deviations corresponds to S_a and S_b , respectively.

Limit of detection, LOD , is the lowest amount that can be detected in a sample, whereas limit of quantification, LOQ , is the lowest amount that can be quantified with acceptable accuracy and precision. These limits are calculated by Equations D.1.1 and D.1.2.

$$LOD = 3 \times S_b/a \quad (D.1.1)$$

$$LOQ = 10 \times S_b/a \quad (D.1.2)$$

The obtained calibration curve is presented in Figure D.0.2 and is presented in Equation D.1.3.

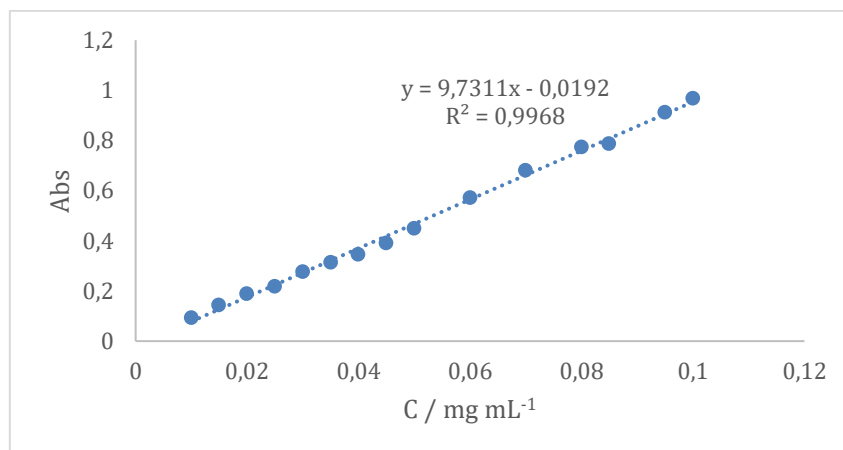


Figure D.0.2 – OP extract calibration curve.

$$Abs = (9.73 \pm 0.33) \times C - (0.02 \pm 0.02) \quad (D.1.3)$$

Where *Abs* represents that measured absorbance and *C* the sample concentration.

Table D.0.1 presents the validation parameters obtained for the calibration curve. All five parameters were verified, and the smallest concentration (0.01 mg mL^{-1}) was superior to both limit of detection and limit of quantification. The curve was considered suitable for use.

Table D.0.1 - Validation parameters for the calibration curve.

Parameter	Calibration Curve
At least 5 different concentrations	15
Concentration range ≥ 10	10
$R^2 \geq 0.995$	0.997
$S_a/a \leq 0.05$	0.02
$b - t \times S_b < 0 < b + t \times S_b$	$-0.04 < 0 < 1.24 \times 10^{-5}$
<i>LOD</i>	$2.74 \times 10^{-3} \text{ mg mL}^{-1}$
<i>LOQ</i>	$9.13 \times 10^{-3} \text{ mg mL}^{-1}$

Appendix E - Sunscreen Formulations

E.1 - Production of Sunscreen Formulations

Sunscreen formulations were produced as described in Section 3.6. Figure E.0.1 illustrates the steps and procedure to produce the base formulation.



Figure E.0.1 - Sunscreen production steps: a- goblets containing phase A; b - Goblets containing phase B; c - Phase A and Phase B in heating bath until dissolution; d – Dissolved phase B; e - Homogenization until the production of a homogenized emulsion.

Appendix F - Absorbance Spectra

Absorbance spectra of each formulation was utilized to calculate the sun protection factor for the three analysis times. Figure F.0.1 illustrates the obtained spectra for the several formulations.

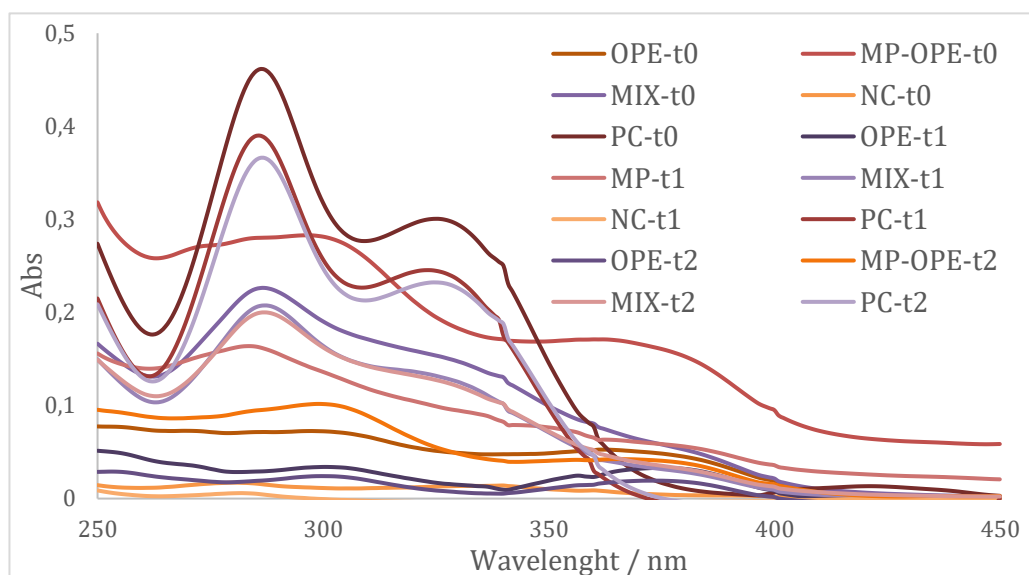


Figure F.0.1 - Absorbance spectra of the different formulations for the three analysis times. *t0* – analysis performed at the week of production; *t1* – analysis performed two weeks after production; *t2* – analysis performed four weeks after production; OPE – Onion Peel extract formulation; MP-OPE – Microencapsulated onion peel extract formulation; MIX – Mixture formulation; NC – negative control formulation; PC – positive control formulation.