Master in Bioengineering

Photoprotective Potential of Avocado and Onion Peels in Sunscreens

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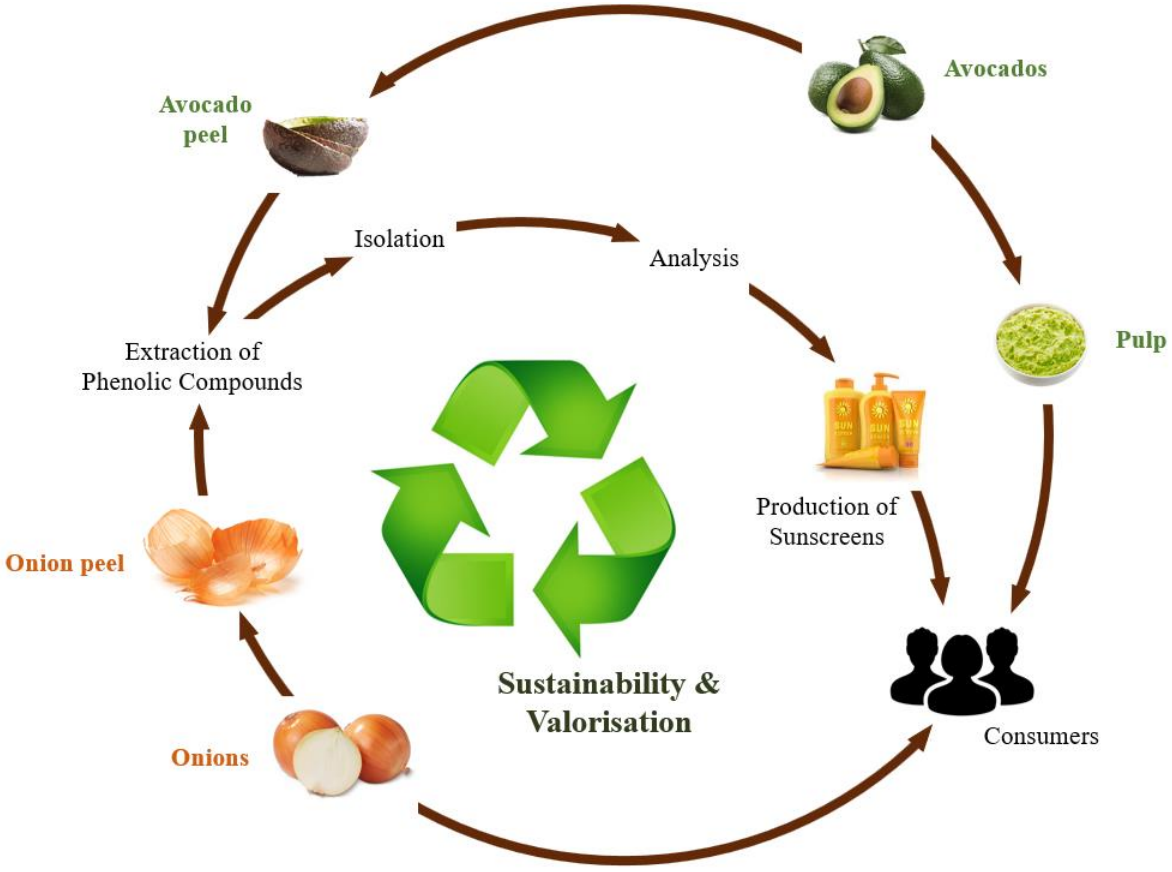
Graphical Abstract



Abstract

Agro-industrial by-products managed as waste represent a serious environmental and economic problem. These residues are at the same time a rich source of bioactive compounds. In the present study, avocado peel extract (APE) of the Hass variety and onion peel extract (OPE) of the brown variety were obtained by Continuous Solid-Liquid extraction (Soxhlet) with ethanol at 70 ºC for 3 h. The antioxidant capacity of the extracts was evaluated with the Folin-Ciocalteu and 2,2-diphenylpicrylhydrazyl (DPPH) assays, that showed that both extracts possess antioxidant capacity, but the OPE exhibited a much higher antioxidant capacity compared to the APE. The OPE also demonstrated strong antioxidant capacity similar to Trolox (positive control). The photoprotective capacity of the extracts was evaluated by determining their Sun Protection Factor (SPF) value. The OPE exhibited a higher SPF value compared to APE and organic UV filter Oxybenzone. An identification of the phenolic compounds (-)-epicatechin and quercetin in the extracts was carried out with a reversed-phase high performance liquid chromatography (RP-HPLC), coupled with a photodiode array detector (DAD), with the mobile phase in an isocratic elution. (-)-Epicatechin, one of the major phenolic compounds found in avocado peels, was identified in the APE with the method but its quantification was not possible due to overlapping spikes, revealing a need to implement a gradient elution to the method. Quercetin, one of the major phenolic compounds found in brown onion peels, was identified, and quantified in the OPE, with a concentration of 107 ± 3 mg g^{-1}, a concentration higher than that found in the literature. The OPE was selected for further evaluation in a cosmetic formulation. Eight different creams formulations as water-in-oil emulsions were produced in order to evaluate the OPEs stability and photoprotective capacity in a sunscreen. The accelerated stability tests performed were physical (centrifugation), thermal (temperature cycling) and oxidative (Peroxide Value). The results demonstrate that there is a need for a more physically and thermally stable base formulation in order to evaluate the OPEs stability in a cosmetic formulation. The Peroxide Value test showed that formulations with OPE incorporated exhibited a similar oxidative stability to that of the formulations with incorporated butylated hydroxytoluene (BHT), the positive control of the test and a common synthetic antioxidant incorporated into cosmetic products. The photoprotective capacity of the formulations was also evaluated by the SPF, in which the formulations with OPE incorporated demonstrated photoprotective capacity and a possible synergetic effect with Oxybenzone in a sunscreen. The present study showed that the APE of the Hass variety possesses antioxidant capacity, and the brown OPE possesses antioxidant and photoprotective capacities, and that, therefore, these agro-industrial by-products display the potential to be harnessed by the cosmetic industry to produce value-added products.

Keywords: avocado peel; onion peel; antioxidant; UV filters; sunscreens.

Resumo

Os subprodutos agro-industriais geridos como resíduos representam um grave problema ambiental e económico. No entanto, estes resíduos são ao mesmo tempo uma rica fonte de compostos bioativos. Neste estudo, extratos de casca de abacate (APE) da variedade Hass e extrato das cascas de cebola (OPE) da variedade castanha foram obtidos por extração Contínua Sólido-Líquido (Soxhlet) com etanol a 70 ºC por 3 h. A capacidade antioxidante dos respetivos extratos foi avaliada com os ensaios de Folin-Ciocalteu e 2,2-difenilpicrilhidrazil (DPPH), que demostraram que ambos extratos apresentam capacidade antioxidante, no entanto o OPE apresentou maior capacidade antioxidante que comparativamente ao APE. O OPE também demostrou uma forte capacidade antioxidante similar ao Trolox (controlo positivo). A capacidade fotoprotetora dos extratos foi avaliada pela determinação do Fator de Proteção Solar (SPF). O OPE exibiu um maior valor de SPF comparativamente ao APE e ao filtro orgânico Oxybenzone. A identificação dos compostos fenólicos (-)-epicatequina e quercetina nos extratos foi realizada por cromatografia líquida de alta eficiência em fase reversa (RP-HPLC), acoplada a um detetor de díodos (DAD), com a fase móvel em eluição isocrática. A (-)-epicatequina, um dos principais compostos fenólicos existentes na casca de abacate, foi identificada no APE, porém sua quantificação não foi possível devido à sobreposição de picos, revelando a necessidade de implementar uma eluição em gradiente ao método. A quercetina, um dos principais compostos fenólicos existentes no OPE, foi identificada e quantificada no OPE, com uma concentração de 107 ± 3 mg g^{-1}, uma concentração maior que a encontrada na literatura. O OPE foi selecionado para ser avaliado em análises seguintes numa formulação cosmética. Oito formulações de cremes diferentes, do tipo água-em-óleo foram produzidas para avaliar a estabilidade e capacidade fotoprotetora do OPE num protetor solar. Os testes de estabilidade acelerados realizados foram o físico (centrifugação), o térmico (ciclos de temperatura) e de oxidação (Valor de Peróxido). Os resultados demostraram que é necessária a produção de uma formulação base com maior estabilidade física e térmica de forma a poder avaliar a estabilidade de uma formulação cosmética com OPE. O ensaio do Valor de Peróxido demonstrou que a formulação com OPE incorporado apresentou estabilidade oxidativa semelhante à formulação com antioxidante sintético (controlo positivo), hidroxitolueno butilado (BHT). A capacidade fotoprotetora das formulações também foi avaliada pelo SPF, no qual as formulações com OPE incorporado demonstraram capacidade fotoprotetora e um possível efeito sinérgico com Oxibenzona. Este estudo mostra que o APE da variedade Hass possui capacidade antioxidante, e o OPE possui capacidade antioxidante e fotoprotetora, e que, portanto, estes subprodutos agro-industriais apresentam potencial para serem utilizados pela indústria cosmética para produzir produtos de valor acrescentado.

Palavras-chave: casca de abacate, casca de cebola, antioxidantes, filtros UV, protetores solares.

Declaration

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

Zizina M. Falé

Porto, 4th of July 2022

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Notation and Glossary

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List of Acronyms

APE – Avocado Peel Extract

OPE – Onion Peel Extract

GHG – Greenhouse gas

FAO – Food and Agricultural Organization of the United Nations

UV – Ultraviolet

ROS – Reactive Oxygen Species

GRAS – Generally Considered As Safe

F-C – Folin-Ciocalteu

FCR – Folin-Ciocalteu's phenol reagent

RSC – Radical Scavenging Capacity (RSC)

DPPH – 2,2-diphenyl-1-picrylhydrazyl

HPLC – High-Performance Liquid Chromatography

DAD – Diode Array Detector

RP – Reversed-Phased

CF – Correction Factor

EU – European Union

USA – United States of America

PV – Peroxide Value

W/O – Water-in-Oil

O/W – Oil-in-Water

1. Introduction

The increase in the numbers of the human population in the last few decades has been accompanied by an increase as well in agricultural wastes generated, which are mostly poorly managed, for example, by burning them or disposing them by inappropriate methods. The environmental impact of the mishandle of these wastes include the continuous emissions of greenhouse gas emissions (GHG) to the atmosphere, which contributes to the pollution of the environment and the warming of the planet. Therefore, properly managing these wastes in an environmentally friendly way is of outmost importance [1].

Two of these wastes are the by-products that result from the processing of the avocado fruit and the onion vegetable, these are the avocado peel and the onion peel or skin. Scientific evidence suggest the existence of bioactive compounds with antioxidant capacities in these wastes, and that their recovery and utilization for the acquisition of value-added products such as phenolic compounds for the food and cosmetics industry is possible, reducing in this way the negative environmental effects that these wastes perpetuate, along with the economic costs of their disposal.

Therefore, the present study aimed at the recovery of avocado and onion peels, obtain extracts from them and perform chemical analysis of these with the Folin-Ciocalteu and 2,2-diphenyl-1-picrylhydrazyl (DPPH) assays, in order evaluate their antioxidant capacity. Another aim of this study is to determine the Sun Protection Factor (SPF) of the extracts in order to evaluate the extracts photoprotective capacity. It was intended also to identify and quantify the main phenolic compounds in these extracts, namely (-)-epicatechin in the avocado peel extract and quercetin in the onion peel extract, using the chromatographic technique reversed-phase high performance liquid chromatography (RP-HPLC), coupled with a photodiode array detector (DAD). And finally, after the characterization of the extracts, the present study also aimed at the production of cream formulations as water-in-oil emulsions with the extracts incorporated in order to determine their stability and photoprotective potential in a cosmetic product.

2. Context and State of the art

2.1. Agro-industrial by-products – the case of avocados and onions

In current times, with the ever more rising numbers of the human population, there is an increase in agricultural production in order to guarantee food security. Agriculture has a critical role in human and economic development; however, its growth and expansion is accompanied by an increase in the amount of food wastes generated. Depending on the product, processing technique and purpose, each one of the agricultural steps - production, processing, distribution and consumption - generates waste [2].

Agricultural waste, co- and by-products are primary residues of plant or animal origin, produced by the agricultural and food processing sectors, which are considered to have no further application. The plant residues include bagasse, seeds, peels, trimmings and bran, are generated in large amounts and have environmental and economic impacts. The environmental impacts of these by-products include the generation of greenhouse gas (GHG) emissions due to microbial biodegradation, leachate treatments and incineration of landfills for fungi and parasite elimination, and the economic impacts are the costs that derive from the handling of these wastes, attributed to landfill management and other waste disposal practices [3–5]. With a world population of 9 billion people expected for 2050, food demand will rise, resulting in an increase in food production and agricultural wastes produced, exerting even more pressure on the environment [3].

Many current initiatives aim to reduce the yearly waste load that results from anthropogenic activities in order to shift to a more circular economy and to achieve sustainability, a concept that has gained momentum in last years [6,7]. The United Nations Environmental Program defines the term Sustainable Consumption and Production (SCP) as the “production and use of goods and services that respond to basic needs and bring a better quality of life, while minimizing the use of natural resources, toxic materials and emissions of waste and pollutants over the life cycle, so as not to jeopardize the needs of future generations” [8].

Two examples of agricultural crops used in the food processing industry that generate by-products treated as waste are avocados and onions, illustrated in Figure 1. The avocado fruit, derived from the avocado tree of botanical name *Persea americana* Mill., is a tropical and subtropical pear-shaped climacteric berry native to Mexico and Central America.

Anatomically it generally has a single large seed surrounded by a thin film of endocarp, an oil-rich pulp called mesocarp, and an outer peel denominated exocarp that can be smooth or rough, thin or thick, and of green and/or purple shades [9,10].

The onion, also known as the common onion, is a vegetable root crop, believed to be original and first domesticated in south-west Asian countries such as Turkmenistan and Afghanistan. It has the botanical name *Allium cepa* L. and is a biennial plant that produces a large single bulb made of ‘’fleshy’’ fresh inner scales and ‘’papery’’ dry outer scales or skins. The onion bulb is a storage organ, where the fresh inner scales store water and nutrients, and the outer dry scales protect the inner scales from damage, moisture loss and fungal attacks. Onions, along with other species from the same genus like the garlic, are well known for their characteristic pungent flavor, which is due to the presence of S-alk(en)yl cysteine sulfoxides, which are compounds that contain sulphur [11–13]. Many different colors of onion peels/skins can be found from white, yellow, purple-red and brown [13].

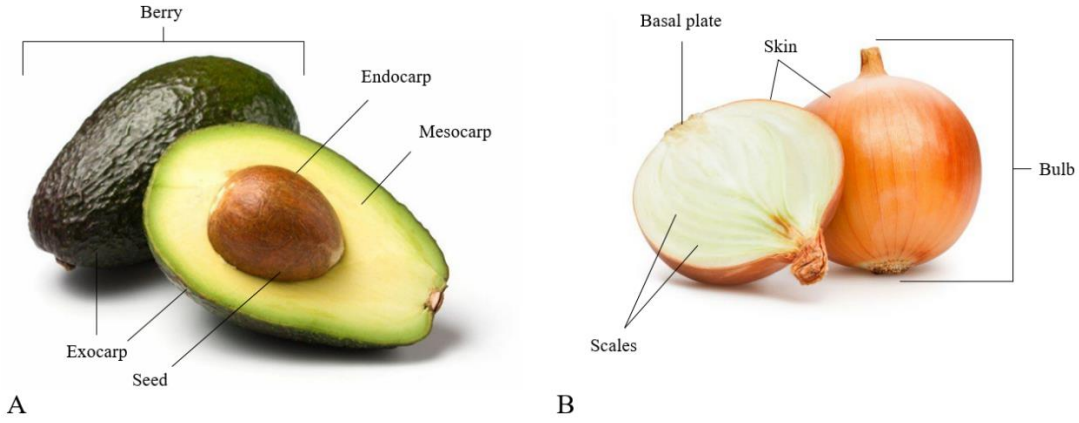

Figure 1 – Anatomy of avocados (A) [14] and common onions (B) [15].

Onions are one of the oldest crops to be cultivated, and one of the most important vegetable species produced worldwide [11], and according to the Food and Agriculture Organization (FAO), in 2020, approximately 105 million tons of dry onions were produced all over the world. Avocados in contrast have a smaller production index, of around 8 million tons for the year 2020 [16], however, popular awareness of the avocados "healthy food" status, sustained by its nutritional value and possible health benefits is causing an increase in its consumption, which in turn is fueling the rise in its production worldwide [17]. The high demand for this tropical fruit has encouraged the production of avocados particularly in Portugal. The Portuguese media has in recent times reported an increase in avocado production areas, specifically in the Algarve region due to its microclimate [18].

Avocados and onions can be consumed in their fresh forms, or they can be processed into a variety of different products. Avocado processing usually results in the production of avocado oil and guacamole from the pulp, but also in the generation of the by-products seed and peel, which compose approximately 33 % of the fruit and currently possess no commercial value. In the case of onions, the processing of the bulb yields canned onions, onion powder and flakes, and the by-products skins, top and bottom parts, roots, and the outer fleshy scales, which compose 38 % of the fresh weigh of the onion, are considered waste [9,19,20].

These by-products, are generated in very large amounts, are managed as solid waste, and their disposal raises environmental concerns. Onion waste is usually composed of the by-products after processing the bulb, but also of unwanted onion bulbs that appear damaged, diseased, malformed or unsized. Feeding this waste to livestock is not appropriate due to its pungent aroma, nor is it incorporating it in the soil as a fertilizer due to the large presence of sulphur containing compounds and fast emergence of the phytopathogenic fungi *Sclerotium cepivorum.* The only remaining option is the disposal of the onion waste in landfills, that incurs high economic costs, in addition to the already recognized environmental negative effects of landfills. The incineration of onion waste is also costly due to the high moisture content of the bulbs and is environmentally damaging due greenhouse gases emissions [12,15,21,22].

In the case of avocados, the peel and seed are usually disposed in landfills. Employing these by-products in animal feed is particularly challenging because avocado peels and seeds spoil and perish easily due to their high-water content, which means affordable conservation methods are necessary for them to be employed as fodder. Moreover, feeding livestock with avocado waste in high amounts is problematic due to the high concentration of phytochemicals in these wastes, which may impart an unpleasant bitter taste and may cause toxicity in high concentrations [14,23].

Therefore, developing sustainable solutions of fruit and vegetable waste management that includes the efficient harness of their full potential is very important, contributing in this way to a more sustainable food sector [24,25]. In recent years, the cosmetic and food industries have been facing noticeable demand for ingredients derived from natural resources [26], and from an economic perspective, the full harness of agro-industrial by-products can be of great advantage [27]. Not only could the use of these waste materials help reduce the environmental impact caused by the activity of these industries, but it can also help them become more cost-effective [28].

2.2. Avocado and onion peels extracts – phenolic compounds and antioxidant capacity

The peel (or skin) of fruits and vegetables is the outermost layer of these horticultural products that acts as a protective barrier against environmental factors, water loss and microbial degradation. Most peels have no consumer interest for consumption due to their unpleasant bitter taste and rough texture, and thus are considered to have no value. However, research has shown that these materials are a rich source of bioactive compounds with evidenced capacities [25,29,30].

The extracts of avocado peels of different varieties have been shown to possess bioactive compounds with *in vitro* antioxidant capacity by scavenging free radicals [31,32], reducing ferric ions [33,34] and breaking radical chain reactions against peroxyl radicals [27]. The avocado peel has also exhibited higher antioxidant capacity compared to the pulp [26,35], but in fruits in general, the antioxidant capacity of the peels and seeds is always higher than that of the pulp. This antioxidant capacity in plant materials is mainly due to the presence of phenolic compounds, shown by the positive correlation usually verified between total phenolic content and antioxidant capacity assays results [34]. However, the phenolic profile of the avocado peel varies greatly with the degree of ripeness of the avocado, as well as the country of origin and growth conditions that the fruit was subjected to [36]. Environmental stresses such as UV radiation also influences the phenolic profiles of fresh fruit and vegetables, with higher exposure levels of UV postharvest increasing the antioxidant activity and phenolic content of fruits and vegetables [37].

Onions peel extracts of different cultivars also exhibit *in vitro* antioxidant capacity [38,39], but photoprotective capacity as well against ultraviolet (UV) radiation [40]. The brown skin/peel of onions has been shown to exhibit higher levels of total phenolic compounds than the more inner parts of the bulb [22].

Phenolic compounds are, by chemical definition, compounds that have one or more hydroxyl groups directly attached to an aromatic ring and are commonly found in plants as esters or glycosides instead of as free compounds. When more than one phenolic hydroxyl group is attached to one or more benzene rings, they are classified as polyphenols [41]. Figure 2 illustrates the classification of phenolic compounds and a representative for each group is displayed.

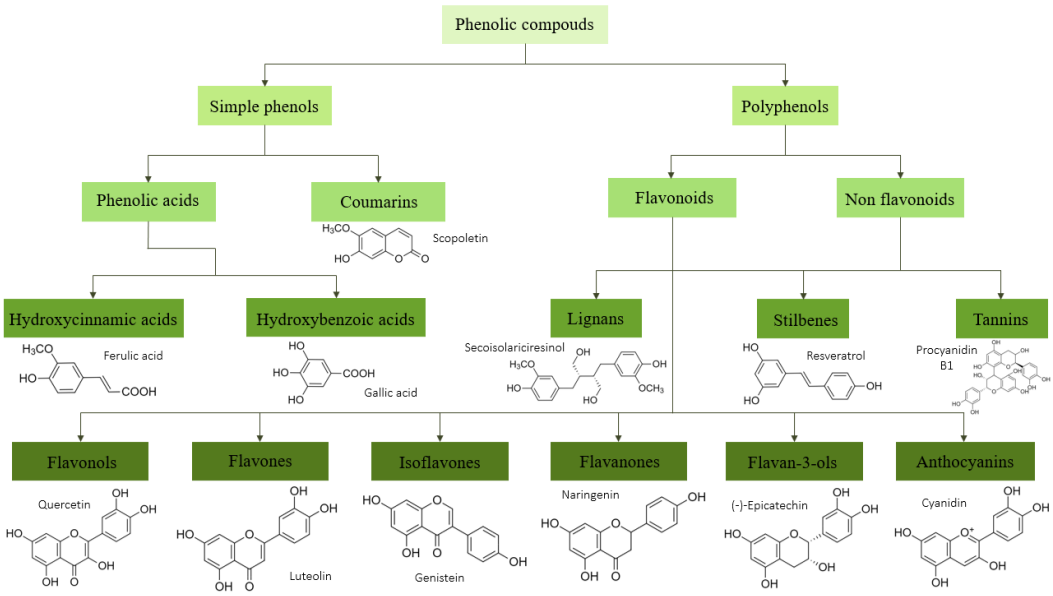

Figure 2 – Phenolic compounds classification and representatives. Adaptation from [42–44] and molecular structures were obtained from PubChem®.

Plants produce phenolic compounds as secondary metabolites during normal development and under stressful conditions. These compounds are the plants defense system against environmental stresses such as light, temperature and UV radiation exposure, and against physiological stresses such as infections and wounds [45,46].

A total of 61 phenolic compounds and other polar compounds present in the extract of the avocado peel have been identified and classified into 11 compound families, with the most abundant ones being procyanidins with diverse polymerization degrees, flavonols, hydroxycinnamic and hydroxybenzoic acids [26]. Epicatechin, a widely distributed flavan-3-ol of the catechin subgroup, and procyanidin B2, a dimer of two epicatechin molecules and one of the most abundant oligomeric flavan-3-ol in fruits, are the major phenolic compounds found in avocado peels [31,33,47].

The flavonol quercetin and its glucosides (mainly quercetin-4'-*O*-glucoside) have been shown to be the major phenolic compounds present in brown onion peel/skin extracts, with increasing concentrations of quercetin and decreasing concentrations of its glucosides towards the outermost layers of the onion bulb [22,48]. This is the result of the maturation and curing process that is performed on onions bulbs post-harvest. Curing is the process of drying the onion bulbs at temperatures above 21 ºC and in a high humidity environment in order to produce one to three layers of dry or semi-dry outer scales, that is, to produce the skin(s). During curing the outer scales of the onion lose water and form the skin that tightly and completely envelops the bulb. This process prevents the inner scales from losing water and

blocks pathogens from entering the bulb, but also changes the appearance of the onion, with the formation of a deep and darker skin color and the production of antifungal compounds, provided both by the breaking down of quercetin glucosides into free quercetin [12,13].

The term antioxidant is used for molecules with the ability to prevent the oxidation of compounds, especially lipid compounds. The mechanisms in which antioxidants act is by scavenging oxygen radicals and by acting as reducing, chelating and synergetic agents, oxidizing themselves before or instead of other molecules [49,50]. Antioxidants are necessary to overcome oxidative stress. Oxidative stress can be defined as an imbalance between Reactive Oxygen Species (ROS) and antioxidants, when the level of free radicals is high compared to that of antioxidants [51,52].

ROS contain an oxygen atom and are highly reactive molecules because of their free (unpaired) electron. A few examples include the hydroxyl radical (·OH), the peroxide (O_2^{2-}), the superoxide radical ($O2^{\cdot -}$) and the hydrogen peroxide (H_2O_2), with the hydroxyl radical being the most reactive. These molecules are produced in all organisms in oxidation reactions during normal metabolism, but under environmental stress for example, their numbers increase above normal levels, and they initiate chain reactions. In chain reactions, the reactive radical abstracts an electron from a nearby molecule, and this molecule becomes a new radical molecule which can react with other molecules, causing therefore the chain reaction. When left unchecked, ROS can cause great damage in a living cell namely by reacting with its membranes lipids and proteins and with its DNA [41].

Depending on the mechanism of action, antioxidants can be classified into primary and secondary antioxidants. Phenolic compounds belong to the primary antioxidant classification because they are capable of donating a proton or participating in single electron transfer mechanisms in order to neutralize the free radicals [51]. When the antioxidant inactivates a free radical, it acquires a radical electron, and instead of propagating the chain reaction by transforming itself into another radical molecule, the conjugated bonds present in the antioxidants molecular structure delocalize the radical electron, rendering therefore the antioxidant a relative stability in the presence of the radical electron and, preventing further damage [41]. Phenolic compounds as antioxidants are very efficient in inactivating ROS, can be regenerated and just a small amount of them is necessary to neutralize a large amount of free radicals [51].

2.3. Identification and quantification of phenolic compounds from extracts

The identification and quantification of phenolic compounds in fruits and vegetables is mainly done by liquid chromatography and/or gas chromatography techniques, but spectrophotometric assays which are relatively more simple techniques can be performed as nonspecific methods in order to evaluate the level of phenolic compounds in these materials [53,54].

One commonly performed spectrophotometric assay used to quantify phenolic compounds in plant extracts is the Folin-Ciocalteu (F-C) assay, also known as the Total Phenolic Content assay or Folin-Ciocalteu's phenol reagent (FCR) reducing capacity assay. This technique is a fast, convenient, and robust method based on the electron transfer reaction between the Folin-Ciocalteu's phenol reagent (FCR) that contains tungsten and molybdenum, and the phenolic compounds present in the sample. Under alkaline conditions, the reagent oxidizes the phenolic compounds to phenolates, resulting in a blue molybdenum-tungsten complex that can be measured in a UV-Vis spectrophotometer in the 760-765 nm wavelength range. An intense blue color usually correlates with a higher concentration of phenolic compounds in the sample. The only drawback with the F–C assay is the fact that the reagent does not react exclusively with phenolics. The FCR reacts with compounds that possess an active hydroxyl group like ascorbic acid, reducing sugars, and aromatic amines and amino acids, which are all oxidizable compounds. The method is usually standardized with gallic acid [53–55].The Folin–Ciocalteu method is usually accompanied by another assay that measures the antioxidant capacity of extract samples. Quite a few assays are available and are commonly used for the relative measurement of the *in vitro* antioxidant capacity of phenolic compounds is plant materials, such as the radical absorbance capacity (ORAC) assay, the ferric reducing antioxidant power (FRAP) assay, the Trolox equivalent antioxidant activity (TEAC) assay and the 2,2-diphenylpicrylhydrazyl (DPPH) scavenging assay [55].

The DPPH assay measures the radical scavenging capacity (RSC) of the phenolic compounds present in the sample. It is a simple and easy method to be employed due to DPPH• being a stable free radical that changes color from violet to a light yellow upon its reaction with an antioxidant, as illustrated in Figure 3. The antioxidant capacity can be quantified based on the decolorization of DPPH at around 515 nm, and the more decolorized DPPH is, the higher the concentration of free radical scavengers (antioxidants) in the extract [55,56].

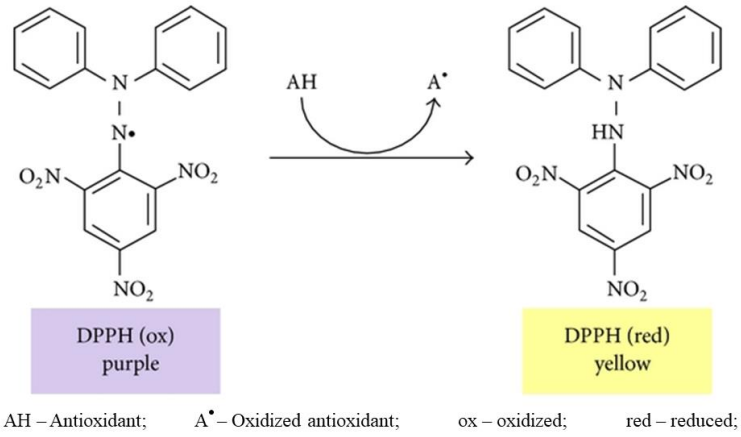

Figure 3 - Illustration of the reduction of the free radical DPPH by an antioxidant [57].

The high-performance liquid chromatography (HPLC) technique is one of the preferred methods for the separation, identification, and quantification of phenolic compounds, where the qualitative and quantitative analytical measurements are made by comparing unknown compounds with standard reference compounds. The most common stationary phase used in HPLC is the C18 reversed-phase (RP) column, that is less polar that the mobile phase, so the phenolic compounds are eluted according to decreasing polarities. RP columns considerably enhance the separation of the phenolic compounds. The mobile phase of an isocratic or gradient elution can be applied, where the gradient elution is normally performed with an aqueous polar solvent such as water and a less polar organic solvent such as acetonitrile or methanol [42,46,53,54].

The detection of the phenolic compounds with HPLC is easily done with fluorescence detectors (FLD), photodiode array detectors (DAD) or with an ultraviolet-visible detector (UV-VIS), where the latter two are the most commonly used [46,54].

2.4. Phenolic compounds from extracts as UV filters in sunscreens

The phenolic compounds that are usually present in plant materials have displayed photoprotective properties and/or synergistic effects on the Sun Protection Factor (SPF) value of cosmetic products when combined with organic UV filters [58–62] as well as inorganic UV filters [63].

The incidence of solar UV radiation in the earth's surface has increased in the last few years due to the depletion of the stratospheric ozone layer [64]. This radiation is divided into three categories according to the wavelength: UVC (100–280 nm), UVB (280–315 nm) and UVA (315–400 nm). The categories that reach the surface of the Earth are only UVB and UVA,

while UVC is dispersed by the ozone-oxygen cycle [65,66], and on average, 20 times more UVA radiation reaches the earth's surface compared to UVB. However, with the depletion of the ozone layer since the 70s, the incidence of UVB rays has increased at the surface, which has correlated with an the increase in the cases of skin cancer in the last few years [64].

The term photoprotection refers to the preventative and therapeutic measures employed to protect the human skin against skin cancer (photocarcinogenisis) and skin aging (photoaging), derived from UVB and UVA radiation exposure, respectively. Photoprotective measures include applying broad-spectrum sunscreens before sun exposure, wearing protective clothing and accessories such as sunglasses and hats, as well as avoiding sun exposure, especially during peak hours of UV radiation [67,68].

Sunscreens of broad-spectrum are products that consist of molecules capable of absorbing, dispersing, or reflecting UVB and UVA radiation. These products have been on the market for many decades now and they have been shown to be effective in preventing skin cancer and aging, dyspigmentation and DNA damage [68,69].

The molecules in sunscreens responsible for the protection against UV radiation are usually referred to as UV filters or Sunscreen agents, and can be categorized as inorganic and organic [70]. It was previously thought that the mechanism of action of inorganic UV filters was by reflecting and scattering UV radiation, while, organic UV filters absorb UV radiation [71]. However, a recent study clarified that inorganic UV filters such as zinc and titanium dioxide actually primarily act as UV-absorbing molecules [72]. UV filters that absorb UV radiation usually possess aromatic rings in their structure with a carbonyl group. They receive the photons from the UV radiation and either undergo conformational changes in their molecular structure, release the incident energy as heat, or emit radiation as a higher wavelength [73].

In the European Union, sunscreens are considered cosmetic products according to legislation [65]. The European Commission defines in the Regulation (EC) No 1223/2009 of the European Parliament and of the Council of 30 November 2009 on cosmetic products, Article 2, that a 'cosmetic product' is "any substance or mixture intended to be placed in contact with the external parts of the human body (epidermis, hair system, nails, lips and external genital organs) or with the teeth and the mucous membranes of the oral cavity with a view exclusively or mainly to cleaning them, perfuming them, changing their appearance, protecting them, keeping them in good condition or correcting body odours" [74]. Europe, along with the

USA, are the largest markets for cosmetic products, with a value of 80 billion euros at retail sale price in 2021, with the skin care sector where sunscreens belong accounting for the largest share as illustrated in Figure 4 [75].

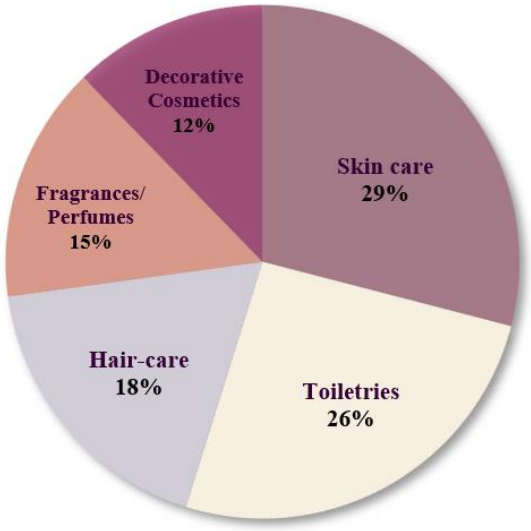

Figure 4 – Shares of the EU market of different categories of cosmetic products in 2021. Adapted from [75].

Sunscreens are usually divided and evaluated for their photoprotective effectiveness according to their Sun Protection Factor (SPF) value and the protection grade of UVA (PA) value. The SPF is defined as the ratio of the amount of radiation needed to produce an erythema with and without a sunscreen topical application [65,71,76].

The efficacy of a sunscreen also depends on the vehicle formulation. Solvents and emollients commonly incorporated in the sunscreen formulations can have a profound effect on the UV absorbance strength. Other ingredients such as emulsifiers determine the uniformity and thickness of the film formed on the surface of the skin, consequently affecting the sunscreens SPF level, its durability and water resistance [65]. In sunscreen formulations, besides the UV filters with photoprotective compounds, compounds with antioxidant capacity and moisturizing compounds are also important [77].

Sunscreens can also be Oil-in-Water (O/W) or Water-in-Oil (W/O) emulsions [78]. An emulsion is basically a stable mixture of two insoluble substances that in normal conditions would separate, but by incorporating an emulsifying agent, they mix and form an emulsion. W/O emulsions are systems in which the internal phase is hydrophilic, containing water, water-soluble and water-miscible substances, whist the external phase is hydrophobic, containing oils, oil-soluble and oil-miscible substances and emulsifier(s)), and vice-versa for O/W emulsions [79,80].

Some of the current challenges in sunscreen formulation are the production of broad-spectrum cosmetics that are photostable and that maintain an unaltered appearance and function when exposed to the UV radiation, as well as the production of a formula that is safe for the individual and that respects the environment [68]. Organic UV filters that are commonly used in sunscreens such as octocrylene, oxybenzone and octyl methoxycinnamate have been surrounded by controversy in last years due to potential harmful effects to human health and the environment. The health concerns are the risk of photoallergy, male and female infertility, endocrine disruption, among others, due to systemic absorption, while the environmental concerns are related to the possible threat that organic UV filters pose to coastal coral ecosystems. However, systemic literature reviews on the effect of organic UV filters show that these have no side effects to human health [68,69], and regarding the environmental concerns, at the moment, there are no environmental exposure data available for many UV filters in coral habitats, and in terms of aquatic toxicity, many of the published studies are of unknown relevance to population-level endpoints, and all contain significant flaws that reduce their reliability and thus suitability for an environmental risk assessment. However, it is too soon to conclude that the present environmental concentrations of UV filters do not adversely impact coral reefs, due to the lack of reliable environmental exposure and toxicity data [81].

Therefore, the present study aimed at the recovery of avocado and onion peels and the extraction of the phenolic compounds in these matrixes by Continuous Solid-Liquid Extraction with ethanol. Afterwards, the present study was divided into two parts: (1) in this first part, the aim was the analysis of the obtained extracts, more specifically their antioxidant capacities by the total phenolic content with the Folin-Ciocalteu assay, and the radical scavenging capacity with the DPPH assay. Also, an evaluation of the photoprotective potential of the extracts by measuring their SPF value, and finally the identification and quantification of the main phenolic compounds found in these extracts, namely (-)-epicatechin in the avocado peels and quercetin in the brown onion peels by RP-HPLC-DAD; (2) The second part of the present study compromised the incorporation of the extracts in cosmetic formulations depending on the performance of these extracts in the first part of the study. This second part also included an analysis of the stability of these formulations, more specifically their thermal, physical, and oxidative stabilities, and lastly an evaluation of their photoprotective capacity as potential additives for sunscreens.

3. Materials and Methods

The following subsections describe the reagents and solvents used in the present study, the extraction method performed, as well as the analysis performed on the extracts and formulations.

3.1. Chemical Reagents

The solvents ethanol (Ref. 83813.360, C_2H_6O, CAS 64-17-5), methanol (Ref. 20864.320, CH_3OH, CAS 67-56-1), acetonitrile (Ref. 83639.320, CH_3CN, CAS 75-05-8) and chloroform (Ref. 22711.290, $CHCl_3$, CAS 67-66-3) were acquired from VWR Chemicals (Fontenay-sous-Bois, France). *Ortho*-phosphoric acid (Ref. AC11001000, H_3PO_4, CAS 7664-38-2) was purchased from Scharlab (Barcelona, Spain). The standard reagents Folin-Ciocalteu's phenol reagent (Ref. 1.09001.0100, CAS 12111-13-6) was acquired from Merck Millipore (Darmstad, Germany), 2,2-Diphenyl-1-picrylhydrazyl (DPPH) (Ref. D9132-1G, $C_{18}H_{12}N_5O_6$, CAS 1896-66-4), (±)-6-Hydroxy-2,5,7,8-tetramethylchromane-2-carboxylic acid (Trolox) (Ref. 238813-1G, $C_{14}H_{18}O_4$, CAS 53188-07-1), quercetin (Ref. Q4951-10G, $C_{15}H_{10}O_7$, CAS 117-39-5), gallic acid (Ref. 91215-100MG, $(HO)_3C_6H_2CO_2H$, CAS 149-91-7) and (-)-epicatechin (Ref. E1753-1G, $C_{15}H_{14}O_6$, CAS 490-46-0) were acquired from Sigma-Aldrich (Riedstraße, Germany), oxybenzone (Ref. A17662, $C_{14}H_{12}O_3$, CAS 131-57-7) was purchased from Alfa Aesar (Zeppelinstraße, Germany), and butylated hydroxytoluene (BHT) (Ref. 47168, C15H24O, CAS 128-37-0) was purchased from Sigma Aldrich (St. Louis, MO, USA). A Merck Millipore Mill-Q water purification equipment, with 18.2 Ω of electric resistance (Billerica, MA, USA), used for deionized water. For the oxidation test, barium chloride dihydrate (Ref.217565, $BaCl_2.2H_2O$, CAS 10326-27-9) and iron chloride III (ref. F2877, $FeCl_3.7H_2O$, CAS 10025-77-1) were obtained from Sigma Aldrich (St. Louis, MO, USA), iron sulphate (II) heptahydrate (Ref. 24244.232, $FeSO_4 \cdot 7H_2O$, CAS 7782- 63-0) and hydrochloric acid (Ref. 20255.290, HCl, CAS 7647-01-0) were bought with VWR (Fontenay-sous-Bois, France), ammonium thiocyanate (Ref. A10632, CH4N2S, CAS 1762-95- 4) acquired from Alfa Aesar (Haverhill, MA, USA), and hydrogen peroxide solution 30 % (Ref. 412072, H_2O_2, CAS 7722-84-1) was purchased from Carlo Erba Reagents (Val-de-Reuil, France).

The cosmetic ingredients Prunus Amygdalus Dulcis (Sweet Almond) Oil, Glycerol, Refined Shea (Butyrospermum Parkii) Butter and Cera Alba (Beeswax) in pearls were purchased from Gran Velada (Zaragoza, Spain), Span® 80 (Ref. S6760-250ML, $C_{24}H_{44}O_6$

CAS 1338-43-8) was purchased from Sigma-Aldrich (Riedstraße, Germany) and glycerol monostearate (Ref. 11324918, $C_{21}H_{42}O_4$, CAS 31566-31-1) obtained from Thermo Fisher Scientific (Kandel, Germany).

3.2. Avocado and onion samples pre-treatment

The onion peels of the brown/yellow onion variety were collected from an agricultural farm in Chaves municipality, Portugal, and the avocado peels were acquired from a local brunch restaurant in the city of Porto, and were identified as being originated from Peru, of the Hass variety, and were visually identified as being ripe, with a dark purple color as it is possible to observe on Figure A1 appendix A.

The samples were manually washed with water and dried with a paper towel. These were then left to dry at 50 ºC in a WTB Binder B115 laboratory drying oven from BINDER GmbH (Tuttlingen, Germany) for 4 h to remove excess water, milled with a Qilive Q.5321 from Auchan (Villeneuve d'Arcq, France) to particles from sizes between 7 to 50 mesh (between 0.297 and 2.83 mm) for the yellow onion peels and 18 to 50 mesh (between 0.297 and 1 mm) for the avocado peels, stored in a desiccator at room temperature and covered with paper foil.

3.3. Extraction by Continuous Solid-Liquid Extraction (Soxhlet)

In order to obtain the avocado peel extract (APE) and onion peel extract (OPE), continuous solid-liquid extractions with a Soxhlet apparatus were performed in triplicate as illustrated in Figure A2 appendix A. The extraction procedure followed the protocol of Ferreira *et al*. (2021) [82], with slight modifications. Ethanol was used as a solvent and a mass-solvent ratio of 1:40 (m/V) as used. Extraction time was 3 h at a temperature of 70 ºC.

Extracts were later evaporated with a rotary evaporator Rotavapor R-200 coupled to a Vacuum pump V-700 and Vacuum controller V-850 (BUCHI Laboratories, Flawil, Switzerland) with a bath temperature of 40 ºC and a pressure of 58 mbar. The samples were then dried with a gentle stream of nitrogen gas to remove residual ethanol.

3.4. Total Phenolic Content of the APE and OPE by the Folin-Ciocalteu assay

In order to determine the Folin-Ciocalteu phenol reagent (FCR) reducing capacity, or Total Phenolic Content (TPC) of the APE and OPE, the Folin-Ciocalteu (F-C) assay was performed following the protocol of Silva *et al*., (2007) [83].

In order to express the obtained results in gallic acid equivalents, a gallic acid calibration curve was prepared from a gallic acid standard in ethanol between the 0.5 to 10 mg L^{-1} concentration range. In a plastic cuvette, 20 µL of gallic acid standard solution or sample, 100 µL of Folin-Ciocalteu phenol reagent and 1580 µL of water were added and mixed well by pipetting the solutions up and down a few times. Afterwards, 300 µL of a saturated sodium carbonate solution of 333.3 g L^{-1} was added and the resulting solutions (triplicates) were left in the dark for 2 h. The absorbance was read at 750 nm with a Thermo GENESYS ™ 10UV UV-Vis Spectrophotometer against a blank solution containing 20 µL of water instead of 20 µL gallic acid standard solution. The concentrations of the APE and OPE were 10 mg L^{-1} in ethanol as well. The gallic acid calibration curve is exhibited in appendix B.

3.5. Radical Scavenging Capacity of the APE and OPE by the DPPH assay

In order to evaluate the Radical Scavenging Capacity (RSC) of the APE and OPE, a 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay was performed following the microplate method described by Bobo-García *et al*., (2015) [56]. A 96-well microplate illustrated in Figure 5 was used to perform the antioxidant analysis.

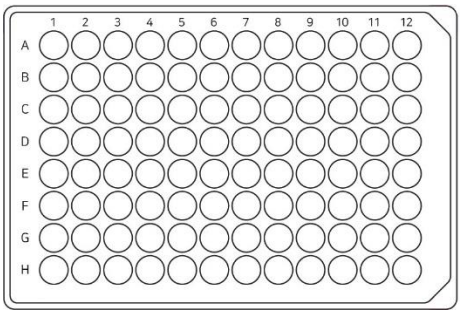

Figure 5 – Illustration of a 96-well microplate.

In order to express the radical scavenging capacity results in Trolox equivalents antioxidant capacity (TEAC), a Trolox calibration curve based on the percentage of DPPH inhibition of Trolox standards was prepared with concentrations between 1 and 10 mg L^{-1}, illustrated in appendix C. DPPH inhibition percentage curves for APE and OPE were also prepared. All of the solutions were prepared in methanol:water (80:20 V/V). 20 µL of

sample/standard was made to react with 180 μL of a DPPH solution of 150 μmol L^{-1} and left to incubate for 40 minutes in the dark, and the absorbance was read at 515 nm in the computer software Gen5 (Agilent Bio Tek). The blank was 20 μL of water with 180 μL of methanol:water (80:20 V/V), and the control was 20 μL of water with 180 μL of DPPH solution. The radical scavenging capacity results of the APE and OPE were also expressed with the half maximal inhibitory concentration (IC_{50}) value as explained in appendix C.

3.6. Photoprotective capacity of the APE and OPE by the Sun Protection Factor assay

In order to determine the photoprotection capacity of the avocado and onion peel extracts, the Sun Protection Factor (SPF) of these extracts was determined according to [84], with slight modifications. Ethanolic solutions of 0.2 mg mL^{-1} for the APE extract and 0.02 mg mL^{-1} for the OPE extract were prepared and the respective absorption values were measured in a UV-3100PC Spectrophotometer by VWR (Darmstadt, Germany), between 280 and 400 nm with a 1 cm optical path length quartz cell. A 0.02 mg mL^{-1} ethanolic solution of (2-Hydroxy-4-methoxyphenyl)-phenylmethanone (oxybenzone) was also prepared and used as a positive control. The SPF values were calculated using equation A1 in annex A.

3.7. Pre-liminary identification and quantification of (-)-Epicatechin and Quercetin in the APE and OPE by RP-HPLC-DAD

The identification and quantification of (-)-epicatechin in the APE and quercetin in the OPE followed the HPLC procedure by Boligon *et al.*, (2015) [85] with slight modifications. The analysis was performed in a Merck Hitachi Elite LaChrom (Tokyo, Japan) high-performance liquid chromatograph, equipped with a Hitachi L-2200 autosampler, L-2130 pump and L-2455 diode array detector. The standards and samples were injected in a Puroshper® STAR RP-18 endcapped LiChroCART® (250 mm x 4.0 mm, 5.0 μm) chromatography column purchased from Merck KGaA (Darmstadt, Germany). An isocratic elution was carried out with the mobile phase consisting of water:acetonitrile (7:3 V/V) with 0.5 % o*rtho*-phosphoric acid, at a rate of 0.8 mL min^{-1}.

The pre-treated avocado and onion peels were contaminated with standards (-)-epicatechin and quercetin, respectively, before the extraction procedure (spiking). The standards and samples were dissolved in acetonitrile:water:ethanol (2:1:1 V/V/V) and filtered through 0.2 μm polytetrafluoroethylene (PTFE) filter and added to 1 mL vials. Identification of the phenolic compounds was done by the retention time (t_r) and quantification with the area

under the curves for quercetin at 365 nm and for (-)-epicatechin at 220 nm. Calibration curves for (-)-epicatechin and quercetin were obtained with standards.

3.8. Production of sunscreens

Due to the results obtained in the Sun Protection Factor determination, only the OPE was selected to be further analyzed in a cosmetic formulation, and in order to evaluate its photoprotective capacity in a sunscreen, eight formulations with the composition exhibited in Table E1 appendix E were produced. The formulations differed in the amount and type of additive incorporated, as displayed in Table 1.

Table 1 – Different amount and types of additives used to obtain formulations F1 to F8.

Formulation	Characteristic	Function
F1	No additives	Negative control
F2	0.5 % BHT	Positive antioxidant control
F3	5 % oxybenzone	Positive SPF control
F4	0.5 % onion peel extract	Sample
F5	5 % onion peel extract	Sample
F6	10 % onion peel extract	Sample
F7	0.25 % BHT + 0.25 % onion peel extract	Sample
F8	5 % oxybenzone + 5 % onion peel extract	Sample

BHT – Butylated hydroxytoluene; SPF – Sun Protection Factor.

Formulations F1 to F8 were prepared as W/O emulsions. First, the aqueous phase ingredients illustrated in Table E1 appendix E were weighted into a glass cup and heated to 70 ºC in a hot water bath. The same procedure was performed for ingredients of the oil phase. After both phases reached 70 ºC, the aqueous phase was added to the oil phase in small amounts, stirred and homogenized with a T 18 digital ULTRA-TURRAX® with stirring speeds of 24000 rpm until full homogenization was visually verified, usually after 5 minutes of stirring. For the formulations F2 to F8, these were left to cool until 40 ºC, the additives were then added, and the stirring and homogenization resumed for another 5 minutes. The formulations were finally left to cool until room temperature and stored at this temperature until further use and evaluation.

3.9. Sunscreen formulations stability tests

In order to evaluate the stability of the formulations produced, three stability tests were performed: thermal, physical, and oxidative. The thermal and physical stability tests followed the protocol of Navarro-Perez *et al*. (2011) [86], with slight modifications.

3.9.1. Accelerated physical stability test: Centrifugation

For the physical stability evaluation, all of the eight formulations were subjected to centrifugation with a 5424 Centrifuge by Eppendorf at 3000 rpm for 10 minutes. Physical stability was evaluated by visually verifying if phase separation occurred.

3.9.2. Accelerated thermal stability test: Temperature variation

An accelerated thermal stability test was performed in all of the eight formulations produced. The planned procedure was: first a high temperature phase by subjecting the formulations to an overnight incubation at 50 ºC, followed by a 20 ºC resting phase during the following day, then a low temperature phase with an overnight incubation at 5 ºC, and finally another resting phase the next day at 20 ºC. Thermal stability was evaluated by visually verifying the existence of phase separation.

3.9.3. Oxidative stability: Peroxide Value test

In order to evaluate the formulated emulsions oxidative capacity, the International Dairy Federation (IDF) protocol, section 74A:1991 [87] method was used, which is an analytical colorimetric method used to determine the Peroxide Value of food lipids. In this method, Fe^{2+} ions are oxidized to Fe^{3+} by hydroperoxides and the reaction of Fe^{3+} with colorless ammonium thiocyanate results in the formation of the colorful complex Fe(III)-thiocyanate, which can be detected in a UV-Vis spectrophotometer [88,89].

First, a Ferric iron calibration curve was prepared in order to obtain the slope of the curve that is used in equation F1 appendix F and in order to express the results in milliequivalents of peroxide per kilogram of formulation.

The Peroxide Value test was performed on the formulations 5 days after their production. Before the analysis of the formulations, samples of these were frozen at -22 ºC in order to destabilize the system, restored to room temperature, centrifuged for 20 minutes at 40.000 rpm and the oil phase separated and stored for the Peroxide Value test. Approximately 0.2 g of the oil phase of the each formulation was dissolved in 9.8 mL of the solvent mixture chloroform:methanol (7:3 V/V), 30 µL of ammonium thiocyanate aqueous solution 300 $mg \cdot mL^{-1}$ was added and vortexed, 30 µL of ferric chloride aqueous solution of 2 $mg \cdot mL^{-1}$ was

added, vortexed and after 5 minutes the absorbance of the solutions in triplicate were measured with a Thermo GENESYS ™ 10UV UV-Vis Spectrophotometer at 510 nm.

3.10. Photoprotective capacity of the formulations by the Sun Protection Factor assay

In order to determine the *in vitro* SPF value of the formulations, the procedure was similar to the one described in section 3.6. Formulation solutions of 0.2 mg mL^{-1} were prepared in absolute ethanol and the absorption values obtained with a spectrophotometer. The SPF values were calculated using equation A1 in annex A.

3.11. 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.

3.12. Conditioning, treatment, and disposal of wastes

The generated liquid waste, which consisted mostly of organic solutions with ethanol and methanol for example, was collected in closed containers, properly identified, and stored away from light and possible sources of ignition and then forwarded to the permitted waste treatment by EcoFEUP (FEUP Environmental Management System).

4. Results and Discussion

4.1. Total Phenolic Content and Radical Scavenging Capacity of the APE and OPE

In Table 2 are exhibited the results obtained in the TPC and RSC assays for the APE and OPE.

Table 2 – Total Phenolic Content and Radical Scavenging Capacity results of the APE and OPE.

	TPC ($mg_{GAE}\ g^{-1}_{extract}$)	DPPH RSC	
		RSC ($mg_{TEAC}\ g^{-1}_{extract}$)	IC_{50} ($mg_{extract}\ L^{-1}$)
APE	86.3 ± 1.4	55.7 ± 1.5	98.1 ± 0.7
OPE	615.1 ± 7.2	826.5 ± 7.1	6.3 ± 0.3
p-value	< 0.05	< 0.05	< 0.05

APE – Avocado Peel Extract; OPE – Onion Peel Extract; TPC – Total Phenolic Content; RSC – Radical Scavenging Capacity; GAE – Gallic acid equivalents; TEAC - Trolox equivalent antioxidant capacity; DPPH - 2,2-Diphenyl-1-picrylhydrazyl.

The gallic acid calibration curve and Trolox calibration curve with both a correlation coefficient (R) of 0.999 can be found in Figure B1 appendix B and Figure C1 appendix C, respectively. The calculations for both assays can be found in appendixes B and C.

Overall, both APE and OPE exhibited antioxidant capacities, however, the OPE displayed greater antioxidant capacity compared to the APE ($p < 0.05$), both in the total phenolic content and DPPH radical scavenging capacity. The IC_{50} value of the OPE was lower than that of the APE, meaning that lower amounts of onion peel extract are needed to provide a DPPH inhibition of 50 %. For comparison, the IC_{50} of Trolox was calculated (not exhibited in Table 2) and was 5.3 ± 0.3 $mg_{TROLOX}\ L^{-1}$, very close to the IC_{50} value of the OPE ($p > 0.05$). Trolox is a vitamin E analog and a very known strong antioxidant, and with these results, it is possible to verify that the extract of the brown onion peel has a very strong antioxidant capacity [90]. The TPC value obtained in the present study for the OPE was much larger that reported in the literature as exhibited in Table 3, and this can be attributed to the difference in extraction techniques employed and origin of the samples.

Table 3 – Comparison of OPE total phenolic content result with the literature.

Study	Extraction methods and conditions	TPC ($mg_{GAE}\ g^{-1}_{extract}$)
Present study	Soxhlet for at 70 ºC for 3 h	615.1 ± 7.2
Benítez *et al*. (2011) [22]	Shaking water bath at 35 °C for 90 min with 70:29.5:0.5 (V/V/V) methanol:water:HCl	52.7 ± 0.9
Benito-Román *et al*. (2020) [91]	Incubator shaker at 37 ºC for 60 min with ethanol:water (70:30 V/V)	46.7 ± 1.4
Lee *et al*. (2014) [92]	Water bath at 60 ºC for 3 h in a with ethanol	372.50 ± 6.85

TPC – Total Phenolic Content; V – Volume.

4.2. Sun Protection Factor of the APE and OPE

For the Sun Protection Factor determination, the results are exhibited in Table 4, and the absorbance spectrums of the APE, OPE and oxybenzone solutions between the wavelengths 280-400 nm can be found in appendix D, Figures D1, D2 and D3, respectively. Ultraviolet absorption peaks were observed for the OPE at 300 and 365 nm, and for oxybenzone at 287 and 325 nm. No absorption peaks were observed for the APE between the wavelength rage analyzed. A drawback of this method is that the SPF values calculated only account for the photostability in the UVB spectrum, while the samples being analyzed may possess UV filter properties in the UVA spectrum.

Table 4 – SPF values obtained for the APE, OPE and Oxybenzone.

Sample	Concentration ($mg \cdot mL^{-1}$)	Sun Protection Factor (SPF)
Oxybenzone	0.02	2
APE	0.2	3
OPE	0.02	5

APE -Avocado Peel Extract; OPE – Onion Peel Extract.

From Table 4, OPE exhibited the highest SPF value, even higher for oxybenzone at the same concentration. With this result, onion peels represent a promising alternative as a UV filter in sunscreens. Regarding the APE, although its SPF value of the in Table 4 is greater than that of oxybenzone, the concentration of APE in the solution had to be 10 times higher than that of oxybenzone and OPE in order to obtain the absorbance spectrum between 0 and 1. Thus, the APE can only provide the same photoprotection to oxybenzone if its concentration is 10 times higher than that of oxybenzone.

As mentioned in the introduction section, (-)-epicatechin is one of the most abundant phenolic compounds in the APE, and quercetin the most abundant in the OPE. The molecular structures of these two phenolic compounds are exhibited in Figure 6 and can justify the SPF results that were obtain.

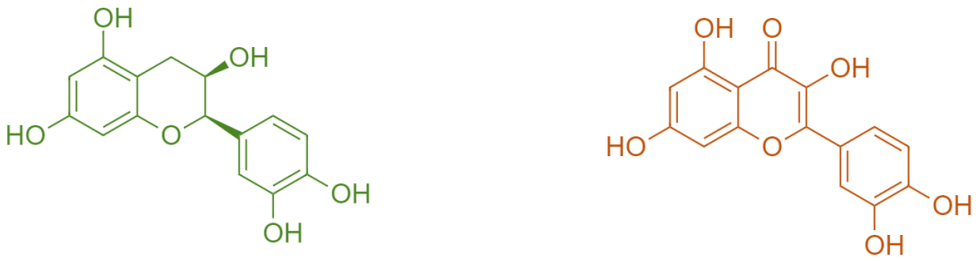

Figure 6 – Molecular structures of (-)-epicatechin (A) and quercetin (B). Structures obtained and adapted from PubChem®.

Both (-)-epicatechin and quercetin are flavonoids, and flavonoids have the general structure composed of a 15-carbon skeleton, containing 2 benzene rings (A and B), linked via a heterocyclic pyran ring (C), as exhibited in Figure 7. The different classes of flavonoids differ in the oxidation level and pattern of substitution of the C ring [93].

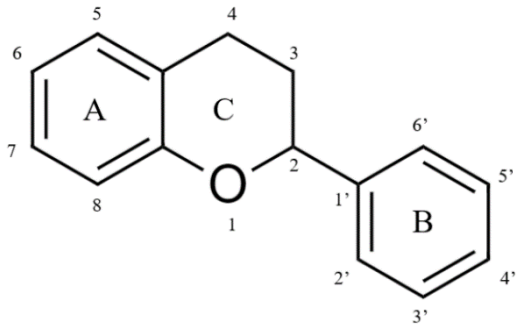

Figure 7 – Basic molecular structure of a flavonoid. Adapted from Kumar *et al*. (2013) [93].

And as mentioned before, chemical compounds that act as UV absorbing agents usually have an aromatic ring and a carbonyl group in their structure. As it is possible to observe from Figure 6, quercetin has a double bond between carbons 2 and 3 on the C ring, conjugated with the 4-carbonyl group also on the C ring, whereas (-)-epicatechin does not. This molecular structure renders quercetin an unsaturated heterocyclic C ring, which promotes a conjugation between the A and B rings [93]. This conjugated nature of the entire molecular structure of quercetin allows it to absorb strongly in the UV radiation [94,95].

Both (-)-epicatechin and quercetin possess the 3',4'-dihydroxy catechol configuration on the B ring, which is a potential radical target, allowing both molecules to have radical scavenging abilities by delocalizing unpaired electrons in the B ring, however only quercetins structure allows from the unpaired electrons to leave the B ring and be stabilized across the hole molecule [96,97]. Not only that, the 3-hydroxyl group also in the C ring in combination with the 2,3 double bond increases the radical-scavenging capacity of quercetin [95].

4.3. Phenolic compounds pre-liminary identification and quantification by RP-HPLC-DAD

The valid calibration curves for (-)-epicatechin and quercetin are exhibited in appendix G.

(-)-Epicatechin was identified in the avocado peel extract (APE) with a retention time of 3.798 min. However, its quantification was not possible due to the presence of another unknown compound overlapping its spike as it is possible to verify in Figure G3 appendix G. In order to quantify this phenolic compound in the APE sample, a gradient elution would have to be applied in order to separate the spikes.

Quercetin was identified in the onion peel extract (OPE), at a retention time of 16.040 min, and quantified with a value of 107 ± 3 $mg \cdot g^{-1}$. Onions are known to be good sources of quercetin [47], and this value is higher than the ones found in the literature [48,98,99], which correlates with the strong antioxidant capacity verified in the previous section. Lee et al. (2014) [92] verified that ethanol allowed for extracts with higher levels of quercetin compared to hot water and subcritical water. The authors Benito-Román (2020) [91] verified that increasing extraction temperatures increased the amount of free quercetin detected using subcritical water in a semicontinuous extractor. However, the authors verified that after the extraction and cooling until room temperature, a high fraction of the extract precipitated. The authors also suggest that extraction at temperatures from 145 ºC contribute to the hydrolysis of the quercetin glucosides to yield more free quercetin.

The retention time values obtained in this study conform with the literature data on the polarity of the two phenolic compounds. The logarithm of the octanol-water partition coefficient of (-)-epicatechin is 0.4 [100], and of quercetin is 1.5 [101], meaning that (-)-epicatechin is a much more polar compound compared to quercetin and, therefore, would be much less retained in the reversed-phase column compared to quercetin.

4.4. Formulations produced

In Figure 6 are illustrated all of the eight formulations produced with different additives incorporated.

Formulations with OPE incorporated exhibited a light brown to dark brown color with higher concentrations of OPE, while the formulations with BHT, oxybenzone and no additives exhibited a white to white-grey color.

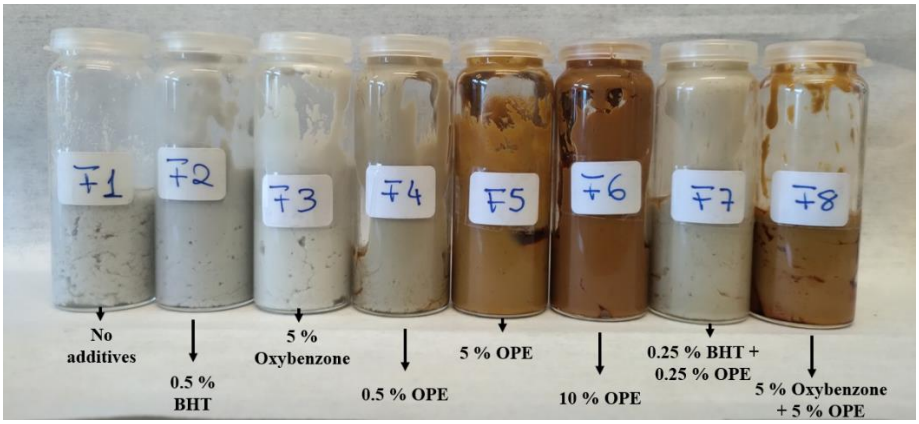

Figure 8 – Formulations F1 to F8 produced with OPE incorporated.

In the following sections, the stability tests results of the formulations are presented.

4.4.1. Stability analysis of the formulations

Figure 9 illustrates the results of the physical and thermal accelerated stability tests performed on the formulations. Figure 9 image A illustrates the formulations after the centrifugation at 3000 rpm for 10 minutes, in which as can be observed, all of the formulations presented phase separation. Figure 9 image B illustrates the formulations after the overnight incubation period at 50 ºC, in which the formulations also presented phase separation. Therefore, the following incubations of the thermal stability test were not performed.

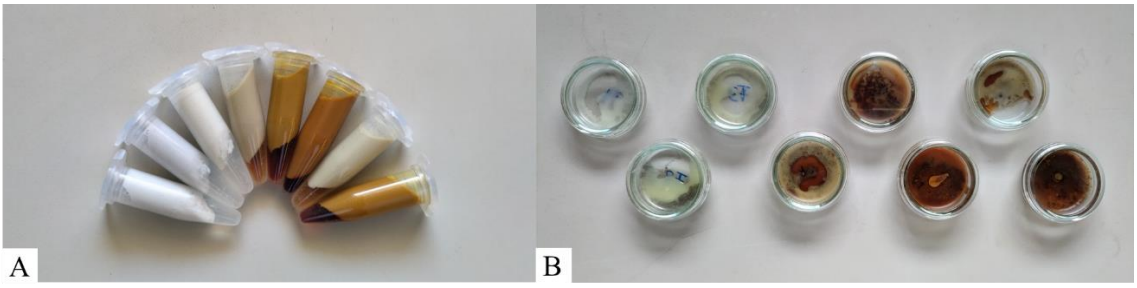

Figure 9 – Formulations F1 to F8 from left to right after the centrifugation (A) and after incubation at 50 ºC overnight (B).

These results show that the base formulation produced did not possess long-term stability. W/O emulsions, compared to O/W emulsion tend to be less stable and because of that have less research done on them, although their emulsion organization allows for more hydrophilic delivery systems which can deliver compounds with antioxidant properties for example [102]. With these results it is not possible to assess if a possible stabilization technique such as microencapsulation is required for the OPE in a W/O cosmetic formulation. Therefore, a W/O

emulsion with greater long-term stability is needed in order to be able to evaluate the stability of the formulations incorporated with OPE.

For the oxidation stability test, Table 5 exhibits the Peroxide Values obtained for the eight formulations. The ferric iron calibration curve is exhibited in Figure F1 appendix F.

Table 5 – Results of the Peroxide Value test on formulations F1 to F8.

Formulations	**Additives**	**Peroxide Value (mEq/kg$_{formulation}$)**	**Oxidation state** [103]
F1	No additives	14.7 ± 0.1	high
F2	0.5 % BHT	12.1 ± 0.4	high
F3	5 % oxybenzone	13.9 ± 1.0	high
F4	0.5 % onion peel extract	11.8 ± 0.5	high
F5	5 % onion peel extract	7.6 ± 0.2	moderate
F6	10 % onion peel extract	6.2 ± 0.1	moderate
F7	0.25 % BHT + 0.25 % onion peel extract	11.3 ± 0.3	high
F8	5 % oxybenzone + 5 % onion peel extract	7.8 ± 0.2	moderate

According to Moigradean *et al.* (2012) [103], the Peroxide Value is the most frequent parameter used to determine the oxidation state of oils and fats. Lipids with Peroxide Values between 1 to 5 mEq kg^{-1} are classified as having a low oxidation state, values between 5 to 10 mEq kg^{-1} are considered to have a moderate state of oxidation, while a value above 10 mEq kg^{-1} is classified as high oxidation state. Looking at the values obtained in Table 5, it is possible to observe that formulations F5, F6 and F8, which were formulated with the highest OPE percentages, exhibited the lowest oxidative state compared to the rest of the formulations. The non-low oxidative state of the formulations in general can be due to the raw materials used in this study, and also the exposure of these to the 70 ºC during the production of the formulations. Nonetheless, these results show that the OPE can help prevent the oxidation of a cosmetic product, rendering it oxidative stability.

Formulation 1 exhibited the highest PV, which was expected due to it being the negative control and not having any compound with antioxidant capacity incorporated. Formulation 3 followed with the second highest PV, no literature data was found on oxybenzone as a chemical compound with antioxidant capacity, and its incorporation in formulation F3 was aimed for its evaluation as a UV filter. The lowest PV was observed for formulation F6 with 10 % OPE, which was expected due to OPE having exhibited a strong antioxidant capacity in the DPPH assay. When comparing formulations F1 and F5, it is possible to observe that the Peroxide Value reduces to half with the incorporation of 5 % OPE, and when comparing formulations F2 and F4, F4 exhibited a close (and slightly lower) PV to F2, which can indicate that the OPE

incorporated in a cosmetic formulation has a similar antioxidant capacity to that of BHT. This is corroborated with the PV value of formulation F7, a combination of BHT and OPE, which displays a PV very similar to the PV of the formulations where just BHT or OPE were incorporated. Therefore, the addition of OPE to cosmetic formulations allows for the same antioxidant protective effect to that obtained when BHT is incorporated.

4.4.2. Sun Protection Factor of the formulations

Regarding to the SPF values of the formulations, the results are displayed in Table 6.

Table 6 – Sun Protection Factor values of the formulations F1 to F8.

Formulations	Additives	Sun Protection Factor (SPF)
F1	No additives	0.02
F2	0.5 % BHT	0.07
F3	5 % oxybenzone	5.2
F4	0.5 % onion peel extract	0.5
F5	5 % onion peel extract	3.0
F6	10 % onion peel extract	6.5
F7	0.25 % BHT + 0.25 % onion peel extract	0.2
F8	5 % oxybenzone + 5 % onion peel extract	9.4

From Table 6, it is possible to observe that formulations F1 and F2 exhibited the lowest SPF values, which was expected due to no UV filter being incorporated in formulation F1 and no literature data being found for BHT as a UV filter. Formulation F3 with 5 % oxybenzone displayed a SPF value of 5.2, which was higher than of formulation F5 with 5 % OPE. These results are contrary to those obtained in the SPF analysis of the extracts, where for the same concentration the OPE exhibited a higher SPF value than oxybenzone. A probable explanation for this can be a reduction in the photoprotective capacity of the OPE when incorporated into cosmetic product. Nevertheless, these results show the photoprotective capacity of the OPE in the UV. From the results of formulations F5 and F6, doubling the percentage of OPE in a cosmetic formulation more than doubles the SPF value of the respective cosmetic formulation. Formulation F8, a mixture of oxybenzone and OPE presented the highest SPF value. When adding the separate SPF values of formulations F3 and F5, the resulting value is less than the one obtained for formulation F8. This result can suggest a synergistic effect when OPE is added to a formulation with oxybenzone. Synergistic effects between plant extracts and synthetic UV filters have been documented in the literature, where the addition of a plant extract containing phenolic compounds increases the SPF value of sunscreen.

It is important to mention that these results only express the capacity of the formulations to inhibit UVB radiation. The UVA range is not accounted for in the equation for the calculation of the SPF values exhibited in Table 6.

5. Conclusions

In this study, avocado peel extracts (APE) and onion peel extracts (OPE) were obtained with a Continuous Solid-Liquid Extraction (Soxhlet) with ethanol at 70 ºC for 3 h. The determination of the total phenolic content (TPC) with the Folin-Ciocalteu assay resulted in values of 86.3 ± 1.4 and 615.1 ± 7.2 $mg_{GAE}\ g^{-1}_{extract}$, for the APE and OPE, respectively. The radical scavenging capacity with the DPPH assay for the APE and OPE resulted in values of 55.7 ± 1.5 and 826.5 ± 7.1 $mg_{TEAC}\ g^{-1}_{extract}$, respectively, and the IC_{50} values were 98.1 ± 0.7 and 6.3 ± 0.3 $mg_{extract}\ L^{-1}$, respectively. With these values, both extracts displayed an antioxidant capacity, however the OPE demonstrated a much higher antioxidant capacity compared with the APE. The OPE also demonstrated a very strong antioxidant capacity due to its IC_{50} value proximity with the IC_{50} value of 5.3 ± 0.3 $mg_{TROLOX}\ L^{-1}$ of Trolox, a very strong commercial antioxidant standard. The Sun Protection Factor (SPF) values of the APE and OPE were 2 and 5, respectively, however, a concentration 10 times higher of the APE solution compared to that of the OPE solution was required in order for it to be measured spectrophotometrically. No peaks of maximum absorption were observed for the APE in the UV radiation spectrum, but two peaks of maximum absorption for the OPE, the first at 300 nm and the second at 365 nm. Hence, the OPE demonstrated a much higher photoprotective potential compared to the APE. The OPE also displayed a greater SPF value compared to oxybenzone (3), a commonly incorporated organic UV filter in sunscreens. The RP-HPLC-DAD analysis allowed for the identification of the phenolic compounds (-)-epicatechin in the APE, one of the main phenolic compounds found in avocado peels, and of quercetin in the OPE, one of the main phenolic compounds found in brown onion peels. However, quantification of (-)-epicatechin was not possible due to overlapping spikes, and therefore an isocratic elution applied to the method is necessary in order to separate the spikes, allowing for the quantification of (-)-epicatechin. Quercetin was quantified in the OPE, with a concentration of 107 ± 3 mg g^{-1}, a very high concentration when compared with the literature. In conclusion, the by-product onion peels exhibited the strongest antioxidant capacity and photoprotective potential for production of sunscreens and therefore followed to the second part of the study, the incorporation phase. The eight formulations produced required improvements in their physical and thermal stability in order to evaluate the stability of the OPE is cosmetic formulations. The oxidation stability test with the Peroxide Value showed that formulations produced with OPE exhibit a similar antioxidant capacity compared to formulations produced with BHT, and the SPF determination of the formulations illustrated that the OPE has a photoprotective capacity and may enhance synergistically the effectiveness of oxybenzone in a sunscreen.

6. Limitations and recommendations for further studies

The results obtained in this study show very promising results for the incorporation of onion peel extracts in sunscreens, however, the amount of time available for the realization of this study was a major drawback, because it did not allow for a more complete study of the capacity and characteristics of the avocado and onion peels. A complete identification of the phenolic compounds in the peels of these by-products extracted with the conditions employed in this study is needed, especially because the onion peel extract exhibited very high amounts of quercetin and a very a high total phenolic content. More time for the development of more stable formulations is needed in order to understand the conditions in which onion peel extracts can be effectively incorporated in sunscreens or other cosmetic formulations, and if techniques such as microencapsulation is needed for its stabilization in emulsions.

The need for the valorization of the by-products derived from the agro-industrial industry is of outmost importance due to the negative environmental effects associated with their disposal. Several studies have shown that different agro-industrial residues exhibit antioxidant capacity, and therefore can be used as a source of antioxidants that can be incorporated into a variety of products. However, the use of the extracts obtained from these by-products must be accompanied by acute, subacute and subchronic 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.

Another aspect that must be addressed is the possible presence of pesticides in the extracts, that result from the use of these during the cultivation of the crops. The identification and quantification of the respective pesticides should be performed in order to guarantee their absence in the extracts.

However, food wastes can also contain potential pathogens, organic residues, high water content, and physical and chemical contaminants that may be a threat to health. Therefore, legislation, regulations and further scientific research are of utmost importance. Besides, the evaluation of safety and quality of valorized by-products must be guarantee as it occurs with the rest of products intended for human or animal consumption [104].

7. References

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Annex A – Calculation of the Sun Protection Factor

The SPF values of the APE and OPE were calculated with equation A1.

$$SPF = CF \times [\sum_{290}^{320} EE(\lambda) \times I(\lambda) \times Abs(\lambda)] \quad \textbf{(Eq. A1)}$$

Where CF in the Correction Factor equal to 10, EE(λ) is the erythemal effect spectrum and I(λ) the solar intensity spectrum for a given wavelength λ, with $EE(\lambda) \times I(\lambda)$ being the normalized product function and with values presented in table B1. Abs(λ) is the absorbance value of the sample at the wavelength λ.

Table A1: Normalized product function in function of the wavelength.

Wavelength (λ) in nm	**EE(λ) × I(λ) (normalized)**
290	0.0150
295	0.0817
300	0.2874
305	0.3278
310	0.1864
315	0.0839
320	0.0180
	Total 1

Appendix A – Avocado and Onion Peels Pre-treatment and Extraction

The illustrations of the materials and methods – extraction section, are here presented.

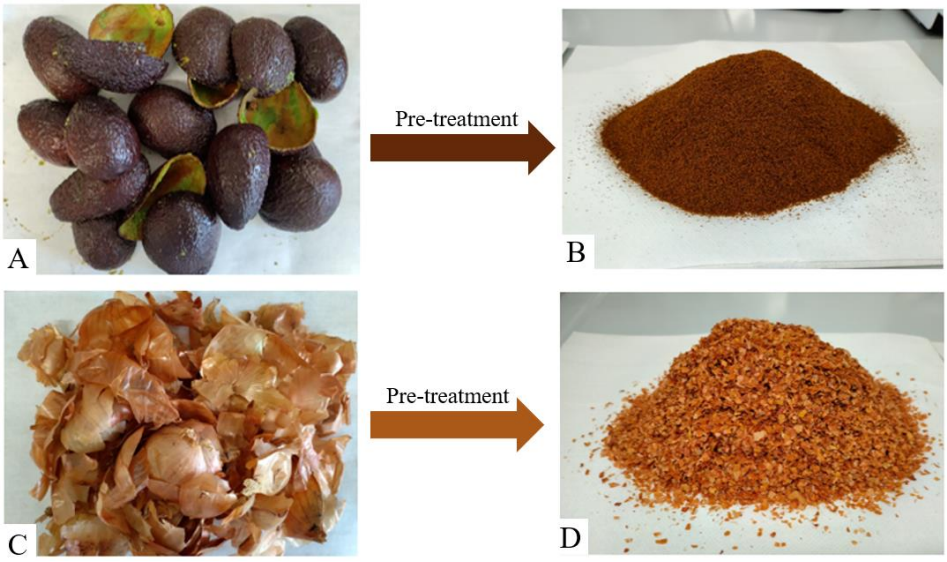

Figure A1 – Avocado peel samples before (A) and after (B) the pre-treatment, and onion peel samples before (C) and after (D) pre-treatment.

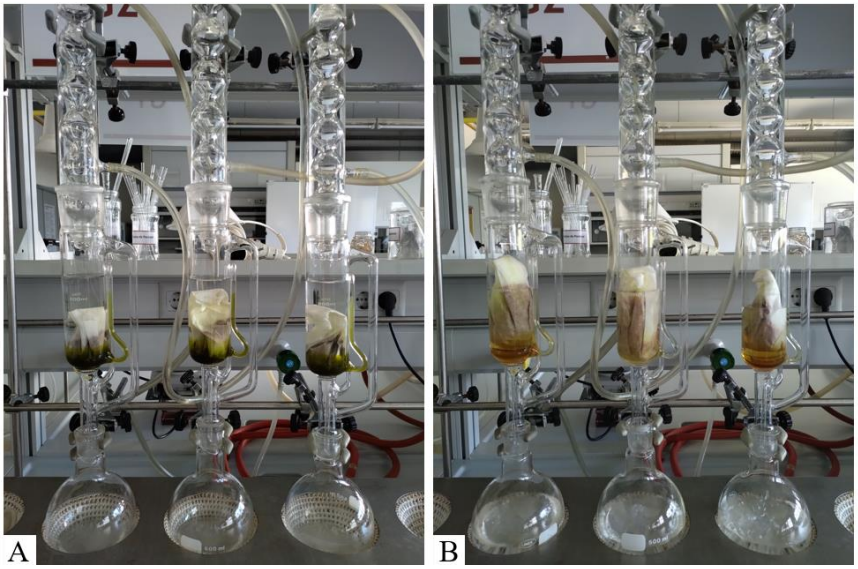

Figure A2 – Soxhlet extraction assembly for the avocado and onion peels (A and B, respectively).

Appendix B – Total Phenolic Content

The gallic acid calibration curve is illustrated in Figure B1, and the corresponding linear regression equation in equation B1, with a Pearsons correlation coefficient (R) of 0.999. The 10 mg L^{-1} standard solution was not included in the calibration curve in order to enhance the R value and still the results of the APE and OPE were within the concentrations of the gallic acid standard calibration curve.

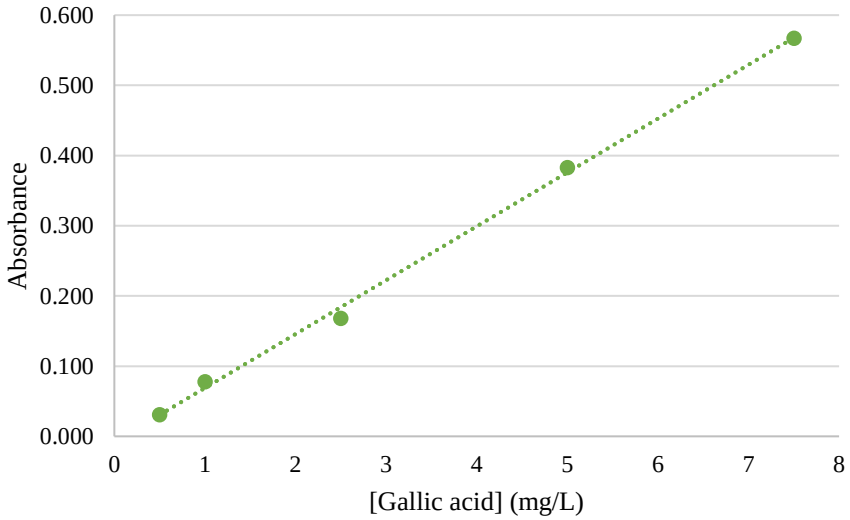

Figure B1 – Gallic acid calibration curve.

$$\text{Abs} = (0.077 \pm 0.003)\left(\text{L mg}_{\text{GAE}}^{-1}\right) \times [\text{Gallic acid}](\text{mg}_{\text{GAE}}\ \text{L}^{-1}) + (-0.008\ \pm 0.012) \qquad \textbf{(Eq. B1)}$$

The APE and OPE total phenolic content values in mg of gallic acid per liter of sample solution were calculated with equation B2, a rearrangement of equation B1, and the corresponding values in mg of gallic acid per g of extract ($mg_{GAE}\ g^{-1}_{extract}$) were calculated with equation B3.

$$\text{TPC}\ (\text{mg}_{\text{GAE}}\ \text{L}^{-1}) = \frac{\text{A}_{\text{sample}} - (-0.008 \pm 0.012)}{(0.077 \pm 0.003)} \qquad \textbf{(Eq. B2)}$$

$$\text{TPC}\ (\text{mg}_{\text{GAE}}\ \text{g}_{\text{extract}}^{-1}) = \frac{\text{TPC}\ (\text{mg}_{\text{GAE}}\ \text{L}^{-1}) \times 1000}{\text{C}_{\text{sample}}(\text{mg L}^{-1})} \qquad \textbf{(Eq. B3)}$$

Where A_{sample} is the measured absorbance value of the extract sample C_{sample} its exact concentration.

Appendix C – Radical Scavenging Capacity

After obtaining the absorbance values of the Trolox standard, APE and OPE, their percentage of DPPH inhibition was calculated using equation C1.

$$\%\ \text{DPPH inhibition} = \frac{\text{A}_{\text{sample/standard}} - \text{A}_{\text{blank}}}{\text{A}_{\text{control}} - A_{blank}} \times 100\ \% \qquad \textbf{(Eq. C1)}$$

Where $A_{sample/standard}$ is the measured absorbance value of a sample or Trolox standard, A_{blank} is the absorbance value of the blank, and $A_{control}$ is the absorbance value of the control, all of them measured after 40 min at 515 nm. The Trolox DPPH inhibition percentage curve is illustrated in Figure C1, and the corresponding linear regression equation in equation C2, with a Pearsons correlation coefficient (R) of 0.999.

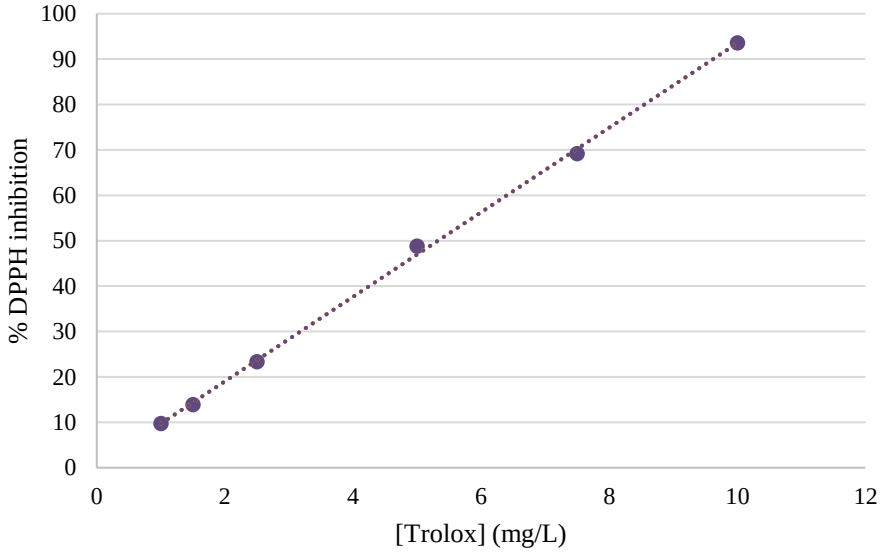

Figure C1 – Trolox calibration curve.

$$\text{Abs} = (9.314 \pm 0.292)\left(\text{L} \cdot \text{mg}_{\text{TEAC}}^{-1}\right) \times [\text{Trolox}](\text{mg}_{\text{TEAC}} \cdot \text{L}^{-1}) + (0.390 \pm 1.646) \qquad \textbf{(Eq. C2)}$$

The radical scavenging capacity of the APE and OPE were expressed Trolox equivalents antioxidant capacity ($mg_{TEAC}\ g_{extract}^{-1}$), following equations C3 and C4.

$$\text{RSC}\ (\text{mg}_{\text{TEAC}}\ \text{L}^{-1}) = \frac{\%\ \text{DPPH inhibition}_{\text{sample}} - (0.390 \pm 1.646)}{(9.314 \pm 0.292)} \qquad \textbf{(Eq. C3)}$$

$$\text{RSC}\ (\text{mg}_{\text{TEAC}} \cdot \text{g}_{\text{extract}}^{-1}) = \frac{\text{RSC}\ (\text{mg}_{\text{TEAC}}\ \text{L}^{-1}) \times 1000}{C_{sample}(\text{mg}\ \text{L}^{-1})} \qquad \textbf{(Eq. C4)}$$

Appendix C (Cont.)

Where % DPPH inhibition$_{sample}$ is the percentage of DPPH inhibition calculated from the absorbance values measured for a specific sample concentration, and C_{sample} is the respective concentration.

The half maximal inhibitory concentration (IC_{50}) values of the Trolox, APE and OPE, expressed as mg of extract per liter of sample ($mg_{extract}$ L^{-1}), were calculated using the linear regression equation obtained from the corresponding DPPH inhibition percentage curve, in which the value 50 was substituted in the ordinate axis term and the abscissa axis term (the IC_{50}) was calculated.

Appendix D – Absorption Spectrums of the extracts

The APE, OPE and Oxybenzone absorption spectrums are illustrated in figures D1, D2 and D3.

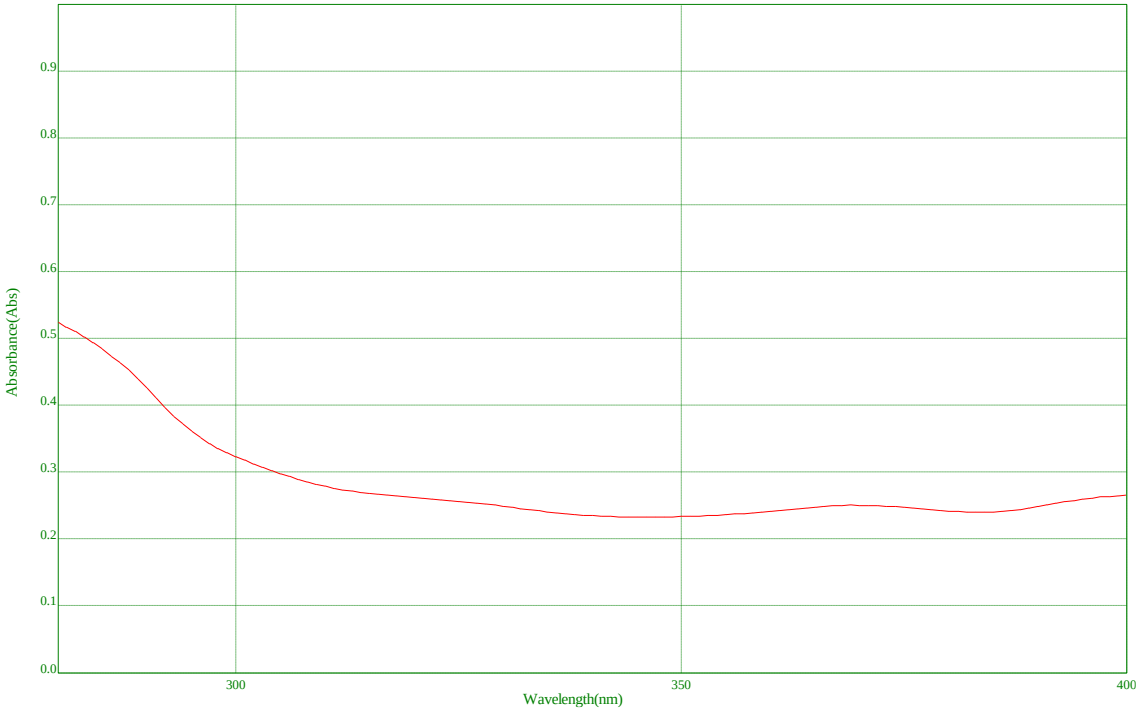

Figure D1: Absorption spectrum of 0.2 mg mL^{-1} avocado peel extract solution.

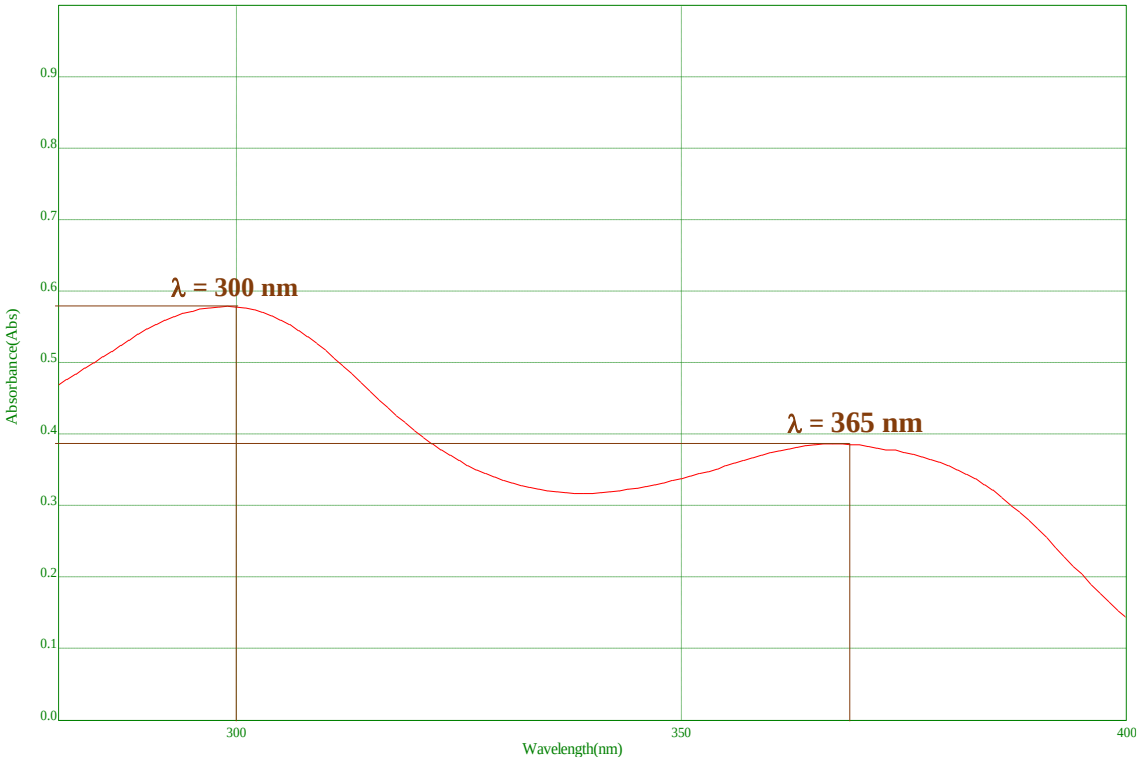

Figure D2: Absorption spectrum of 0.02 mg mL^{-1} onion peel extract solution.

Appendix D (Cont.)

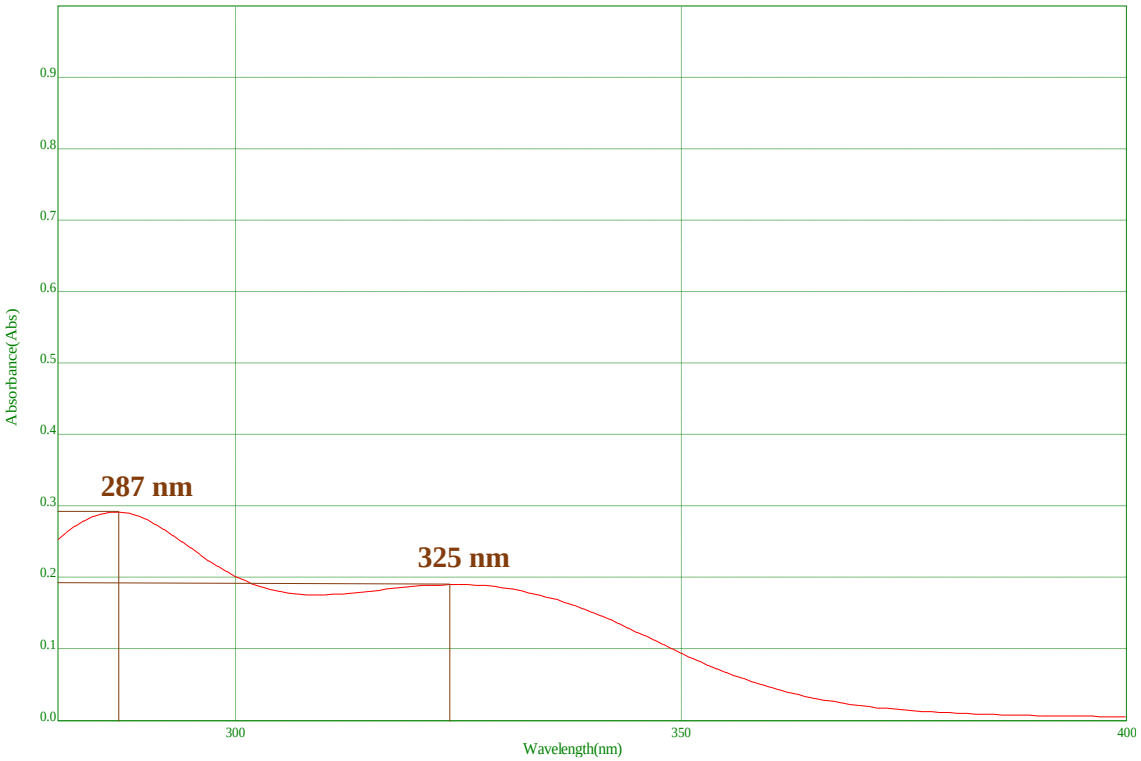

Figure D3: Absorption spectrum of 0.02 mg mL^{-1} oxybenzone solution.

Appendix E – Sunscreen formulation composition

Table E1 - Composition of formulations F1 to F8.

	Percentage (%)							
Sunscreen ingredients INCI or IUPAC name	**F1**	**F2**	**F3**	**F4**	**F5**	**F6**	**F7**	**F8**
Aqueous phase								
Aqua	50	49.75	47.5	49.75	47.5	45	49.75	45
Glycerol	13	12.94	12.35	12.94	12.35	11.7	12.94	11.7
Oil phase								
Butyrospermum Parkii (Shea) Butter	3.5	3.48	3.33	3.48	3.33	3.15	3.48	3.15
Cera Alba (Beeswax)	3.5	3.48	3.33	3.48	3.33	3.15	3.48	3.15
Prunus Amygdalus Dulcis (Sweet Almond) Oil	20	19.9	19	19.9	19	18	19.9	18
Glycerol Monostearate	5	4.98	4.75	4.98	4.75	4.5	4.98	4.5
Span 80®	5	4.98	4.75	4.98	4.75	4.5	4.98	4.5
Additives								
Butylated Hydroxytoluene (BHT) – positive control	-	0.5	-	-	-	-	0.25	-
(2-Hydroxy-4-methoxyphenyl)- phenylmethanone (Oxybenzone) – positive control	-	-	5	-	-	-	-	5
Onion Peel Extract (OPE)	-	-	-	0.5	5	10	0.25	5

INCI - International Nomenclature of Cosmetic Ingredients; IUPAC - International Union of Pure and Applied Chemistry.

Appendix F – Peroxide Value test

Figure F1 illustrates the Fe^{3+} calibration curve obtained when plotting the mass values of ferric iron (Fe^{+3}) *v/s* the absorbance values measured in the spectrophotometer at 510 nm.

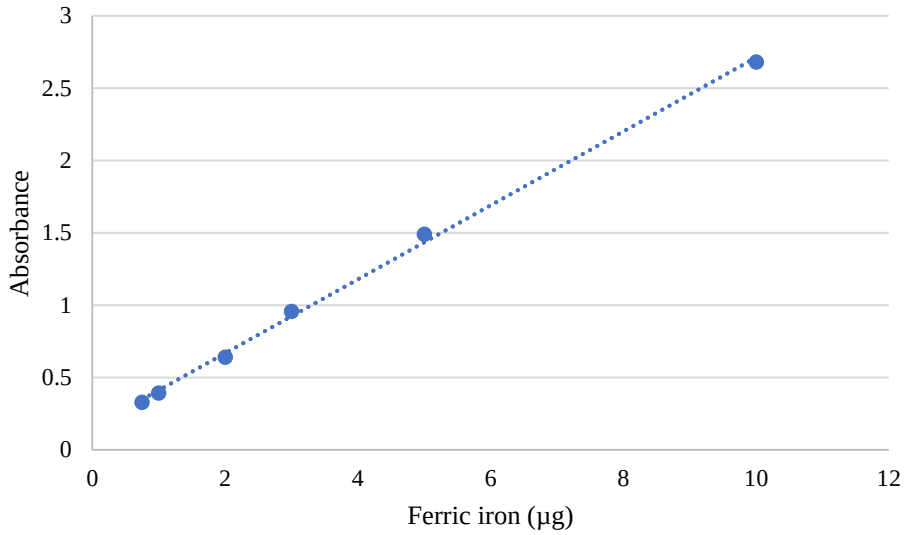

Figure F1 – Ferric iron calibration curve.

The linear regression obtained from the plot in Figure F1 is exhibited in equation F1, with a Pearsons correlation coefficient (R) of 0.999.

$$\text{Abs} = (0.256 \pm 0.008)(\mu g^{-1}) \times \text{Ferric iron } (\mu g) + (0.1557 \pm 0.038) \quad \textbf{(Eq. F1)}$$

The Peroxide Value of the formulations F1 to F8 was calculated with equation F2.

$$\text{PV } (\text{mEq kg}^{-1}_{\text{formulation}}) = \frac{\text{Abs}_{\text{sample}} - \text{Abs}_{\text{blank}}}{\text{m} \times 55.845 \times 2} \times \frac{1}{\text{a}} \quad \textbf{(Eq. F2)}$$

Where $\text{Abs}_{\text{sample}}$ is the mean absorbance value (n=3) of the formulation, $\text{Abs}_{\text{blank}}$ is the absorbance value of the solvent, m is the weight of the sample in kg, and a is the slope of the linear regression obtained from the ferric iron calibration curve, 55.845 is the molar mass of iron and the division by 2 gives milliequivalents of peroxides instead of milliequivalents of oxygen. The Peroxide Value is expressed as milliequivalents of peroxide per kilogram of formulation (mEq $kg^{-1}_{formulation}$).

Appendix G – RP-HPLC-DAD Chromatograms

Figures G1 and G2 exhibit the validated calibration curves obtained in the RP-HPLC-DAD for (-)-epicatechin and quercetin, and equations G1 and G2 their linear regression equations, with R values of 0.999.

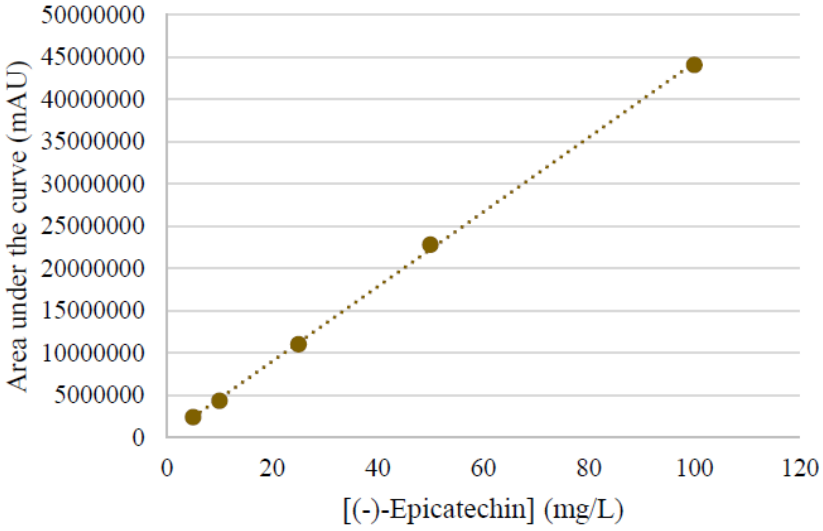

Figure G1 - (-)-Epicatechin calibration curve.

$$\text{Area} = (443091 \pm 7943)(\text{L mg}^{-1}) \times [\text{(-)-Epicatechin}](\text{mg L}^{-1}) + (44841 \pm 345691) \quad \textbf{(Eq. G1)}$$

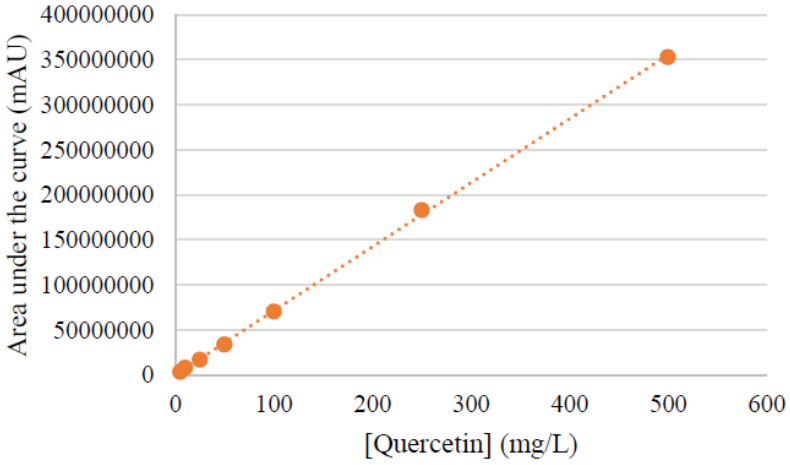

Figure G2 - Quercetin calibration curve.

$$\text{Area} = (710643 \pm 12687)(\text{L mg}^{-1}) \times [\text{Quercetin}](\text{mg L}^{-1}) + (171613 \pm 2736938) \quad \textbf{(Eq. G2)}$$

Appendix G (Cont.)

Figures G3 and G4 illustrate the chromatograms obtained with the reversed-phase high-performance liquid chromatography, coupled with a photodiode array detector (RP-HPLC-DAD), employing a mobile phase of water:acetonitrile (70:30V/V) with 0.5 % *ortho*-phosphoric acid. Figure G3 is the chromatogram of the unspiked avocado peel extract sample, and Figure G4 is the chromatogram of the unspiked onion peel extract sample. Both chromatograms are zoomed in in the retention time of the respective phenolic compound being identified.

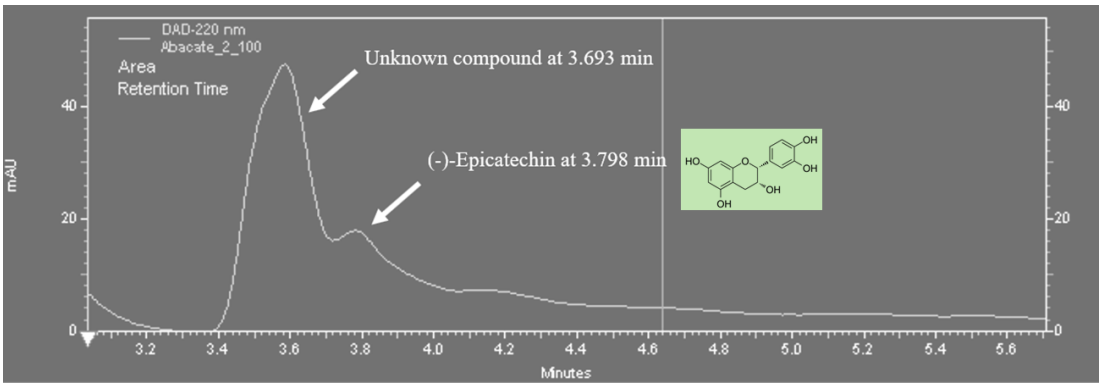

Figure G3 - Zoomed in chromatogram of the unspiked APE sample at λ = 220 nm.

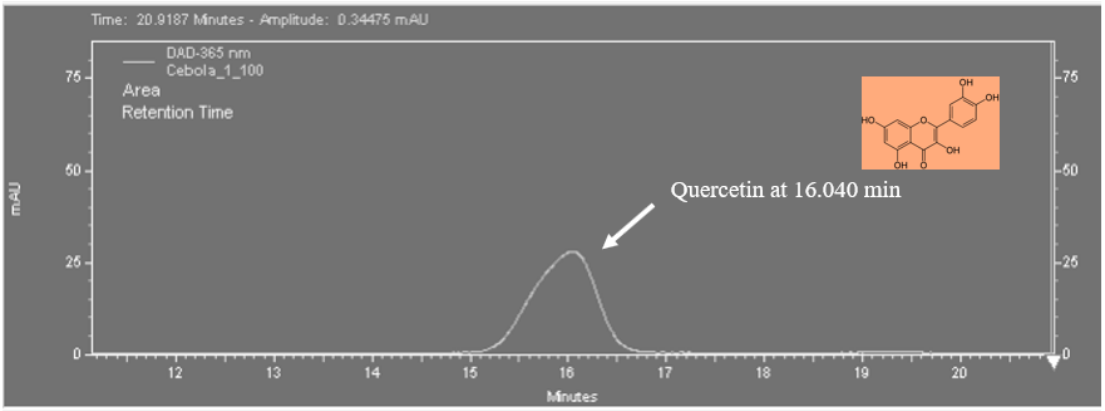

Figure G4 - Zoomed in chromatogram of the unspiked OPE sample at λ = 365 nm.