



Separation of antioxidants from apple (*Malus domestica*) red peels and formulation of a daily face cream

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Abstract

Background: Apples (*Malus domestica*) are a part of most daily diet. Their positive impact on health and overall wellness is recognized and widely accepted. Nowadays, the demanding industry of cosmetology has raised awareness over the potent dangers of the ingredients and consumers tend to reach out for products more nature based and gentle. In this study, the development of different extraction methods for the isolation of antioxidants from apples' peels was tried and the evaluation of their efficacy. In addition to this, it was aimed to formulate a simple daily face cream using this extract and prove its action.

Materials and Methods: This product was tested for its allergens, its stability and its preservative efficacy with the target to conclude to not only an effective daily cream but to a safe product ready to be used. Moreover, the antioxidant activity of the extract was determined as a single extract as well as after its encapsulation in the face cream.

Results: The most common extraction methods were followed and showed that at 200µg/mL concentration the scavenging activity of some extracts is more than 80%. The formulation of those in a product, appeared to have successful results, since the antioxidant value of the peels remained in the product which proved to have a satisfying preservation system, no unforeseen allergens and great stability behavior under different conditions of storage.

Conclusion: This study suggests the extraction of apples' peels and the separation of antioxidant compounds. The findings prove that these kind of ingredients can be used in a product successfully and lead to the daily use by consumers.

Keywords: *Malus domestica*, Emulsions, Chemical analysis, Challenge test, Formulation Antioxidants

Background

During the last years, the annual production of apples (*Malus domestica*) ranges from 10 to 12 million tons within the countries of the European Union. It is considered the fruit with the highest production rate in Europe and its numbers keep increasing. In Greece, apple tree is among the most common cultivations, and the most popular are red varieties like Delicious, Golden Delicious and Granny Smith. Its popularity is linked to its pleasant taste, easy availability and its well-known nutritional character. There are more than 3000 different varieties of apples worldwide, providing a plethora of choices regarding their taste, their organoleptic properties, their resistance to storage etc.^{1,2}

Multiple studies have demonstrated and evaluated the antioxidant capacity of apples. Their nutritional profile includes a list of phenolic compounds that can vary based on the different species of apples in every region. These derivatives belong to secondary metabolites of plants, called often phytochemicals, and they exhibit remarkable

antioxidant activity. The most important groups of polyphenols in apples, have been reported again in the past,³ and they are flavonol glycosides, flavanols (catechin, epicatechin), flavonols (quercetin), procyanidins (anthocyanins), dihydrochalcones and phenolic acids. Actually, apples' skin, which is often considered a by-product of the Food Industry, is the main source of these antioxidants. This is an additional motivation for the research about the potential properties of more natural-based ingredients, since by doing this the natural resources are not deprived. Furthermore, sustainability is becoming a huge issue in most industries including the cosmetics, and by supporting circular economy activities, the benefits are multiple.³⁻¹⁷

Cosmetic industry, one of the most demanding and fast-growing businesses nowadays, is always looking to utilize new materials and innovative ingredients in its products. Companies in their attempt to become more competitive are always looking to find brands that provide natural origin materials, combining proven efficacy along



with compatibility with the needs of the consumers. Public consciousness is raising over the ingredient list of each product and this leads people to seek products that contain safe ingredients, especially these that are inspired by nature or traditional herbal remedies.¹⁷⁻¹⁹

Human skin consists of three major regions, the epidermis, the dermis and the subcutaneous tissue. Keratinocytes are the cell types forming the barrier between skin and the outer environment in the epidermis. Skin aging is mainly mentioned as the leading problem of skin and the center of most of skin problems. It is often connected to environmental reasons, such as pollution, excessive and unprotected sun exposure or low quality of life, which in turn reinforce the production of free radicals. Free radicals are able to cause accumulative oxidative cell damage and become the main contributor to cell aging though damaging DNA. An effective inhibitor in this case are antioxidants. They “scavenge” free radicals by terminating their chain reaction mechanism and neutralizing them by donating an electron. By doing this, they pre-vent damaging vital molecules which can lead to permanent problems thereof.²⁰⁻²²

Emulsions are the most widespread type of cosmetics. They consist usually of two immiscible liquids, in which one is dispersed in the other in the form of droplets. As the dispersed phase, is described the liquid that is in droplets and as the continuous phase, is the liquid in which the dispersed phase is dispersed. The main characteristic that separates emulsions from colloids is the nature of these phases, which in the case of emulsions is liquid. The way to categorize emulsion is mainly based on the nature of each phase. When the continuous phase is consisting of the oily ingredients in which the droplets of the aqueous phase are dispersed, the emulsion is called water in oil (w/o). In contrast, when the dispersed droplets are the oily phase, the emulsion is called oil-in-water (o/w). There are other ways to categorize emulsion such as the nature of the emulsifier, the nature of the oil. Emulsification is the process where under the application of energy, oil and water phase dispense and create one system, the emulsion. In order this to succeed, the system needs one factor that can decrease the surface tension between the two immiscible phases, it is called surfactant.^{19,23-29}

Up to our knowledge, there is a range of studies for the antioxidants and the nutritional value of apples, but without the incorporation of them in a final product. The main product of this project is not only to investigate the most efficient way for the extraction of apple peels but to use them in a product that can easily be a part of everyone's skin care routine. This product should comply with all the necessary quality control guidelines and manage to maintain the antioxidant properties of the extract.

Materials and Methods

Methanol and acetone were purchased by Honeywell

(Seelze, Germany), acetic acid (glacial) 100% (anhydrous for analysis), sodium nitrite solution, sodium hydroxide and 2,2-diphenyl-1-picrylhydrazyl (DPPH) were purchased by Merck (Darmstadt, Germany). L-ascorbic acid, potassium chloride, aluminum chloride and sodium acetate buffer were bought by Sigma-Aldrich (Munich, Germany). Ultra-pure water was purchased by Carlo Erba (Val-De-Reuil, France) and quercetin by Flurochem (Derbyshire, UK). Chemco by Syndesmos (Athens, Greece) provided us with glycerin, *Prunus amygdalus dulcis* (almond) oil, allantoin, D-panthenol and tocopheryl acetate. Sodium phytate and glyceryl stearate citrate were purchased from Evonik Dr. Straetmans (Darmstadt, Germany), potassium palmitoyl hydrolyzed wheat protein (and) glyceryl stearate (and) cetearyl alcohol by Sinerga (Varese, Italy), caprylic/capric triglycerides from Oleon (Ertvelte, Belgium). *Butyrospermum parkii* (Shea) butter, *Macadamia ternifolia* seed oil and *Simmondsia chinensis* (Jojoba) seed oil were bought from Esperis (Milano, Italy) and benzyl alcohol from Thor (Barcelona, Spain). Xanthan gum was provided by Lamberti (Gallarate, Italy), allantoin by Wuhu Huahai Biology (Anhui, China) and cetearyl alcohol by Industria Chimica Panzeri (Bergamo, Italy).

Apples were bought from a local supplier in the central market of Florina (Western Macedonia, Greece). They were carefully chosen based on their integrity.

Extraction

The first extraction protocol (here mentioned as “A”) was based on the method described by Akhtar et al.³⁰ Briefly, the chosen apples were washed with ultra-pure water and carefully inspected. After slicing and milling, the juiced obtained was extracted with a mixture of methanol: formic acid: water (70:2:28). After 30 minutes of continuous magnetic stirring and 2 minutes of homogenization, the mixture is covered with parafilm in a beaker and stayed overnight at 4°C. After that, the mixture is filtered through filter paper and the sediment is washed with methanol: formic acid: water (70:2:28), again. The solvents are removed through rotary evaporation under vacuum, at 45°C. At the end, a portion of water remains with the sample and they are stored at -20°C, until further analysis.

The second extraction protocol, here mentioned as “B”, was based on the method prepared by Reagan-Shaw et al.,²⁵ with slight modifications. For this procedure, apples were washed with ultra-pure Water twice and carefully peeled after drying. The peels were homogenized with deionized Water for about two minutes at high speed. The mixture was then centrifuged at 4000 rpm for 20 minutes at Room Temperature. After the centrifugation, the supernatant was splinted into two parts. The first part (Part B1) was filtered under vacuum using filter with pore size of 0.45 µm and 47 mm diameter, and the second part (Part B2) was filtered through filter paper.

The third and final protocol, was previously described by Wolfe et al.³¹ Apples were washed twice with ultra-pure water, carefully peeled and then the peels were used for extraction for 40 minutes with 80% Acetone. After that, the mixture is homogenized and filtered through Filter paper. Acetone is removed under vacuum with rotary evaporator at 45°C and then it is stored at -20°C until further analysis.

Antioxidant activity

The antioxidant evaluation of the extracts was in vitro evaluated using the DPPH assay, according to the method described by Scalia et al.,³² with slight modifications. 3 mL aliquots of the tested extracts were added to 1 mL of DPPH solution (0.1mM in methanol). The mixture was vortexed for 10 seconds and incubated in the dark at room temperature. After 30 minutes the absorbance of the samples was measured using a UV-VIS spectrometer, at 517 nm. For this experiment, as a control sample a solution with methanol instead of the extract was used and as a standard solution, a solution of ascorbic acid 50 mg/mL.

The DPPH radical scavenging activity of the extracts was calculated based on the following equation (1):

$$\text{Scavenging activity} = \frac{(\text{Control absorbance} - \text{Sample absorbance})}{\text{Control absorbance}} * 100 \quad (1)$$

Where, control absorbance is the absorbance of the control sample and sample absorbance, the absorbance of the tested extracts. Samples were tested in triplicate.

Determination of the flavonoid content

The Flavonoid content of the extracts was measured using a colorimetric method described by Wolfe et al, with minor modifications.³¹ For this experiment a volume of a known dilution of the extract is added with distilled water to a final volume of 2,74 mL, along with 0.075 mL 5% of sodium nitrite solution. This mixture is allowed to stand at room temperature for 5 minutes after it is vortexed. Then, 0.15 mL of 10% aluminum chloride solution is added dropwise and while the solution is in a cold water bath. 6 minutes after this, 1 mL of 1M solution of sodium hydroxide is added and stirred magnetically. A standard curve was made by quercetin solutions in methanol with concentrations ranging from 1 µg/mL to 12 µg/mL. The absorbance of the mixture is measured with a VIS/UV spectrometer at 386 nm and the flavonoid content is expressed as mg quercetin equivalent (QE)/g dry weight (D.W.).

Determination of the anthocyanin content

In order to evaluate the anthocyanin content in the extracts, a spectroscopic pH differential protocol was utilized, as mentioned by Wolfe et al.³¹ For the monomeric anthocyanin content, the extracts were mixed with two

solutions. The first solution is 0.025M potassium chloride buffer at pH 1 diluted with the sample at 10:1 ratio. In parallel, the sample is diluted with sodium acetate buffer at pH 4.5, with the same ratio. The absorbance of the mixtures is determined at 2 wavelengths, 515 and 700 nm, against the blank solution which is distilled water. The anthocyanin content is calculated as described in the equation following (2):

$$\text{Total Monomeric Anthocyanins} \left(\frac{\text{mg}}{100\text{g of fresh peel}} \right) = \frac{A * MW * 1000}{\epsilon * C} \quad (2)$$

Where A is the absorbance: $A = (A_{515} - A_{700})_{\text{pH 1}} - (A_{515} - A_{700})_{\text{pH 4.5}}$, MW is molecular weight for cyanidin 3-glucoside = 26900, and C is the concentration of the buffer in mg/mL.

Formulation of a face cream and in vitro evaluation of its antioxidant activity

In this study, we developed an oil in water (o/w) emulsion containing this antioxidant extract from apple peels along with other ingredients to enhance its ability to protect skin. In order to determine the antioxidant activity of the cream formulation, creams with different concentrations of extract were prepared as well as a base cream formulation where no antioxidant substances were added.

The emulsion was made following the standard formulation procedure. Briefly, the aqueous phase was prepared by adding the ingredients one by one, each after the complete dissolution of the previous. The whole process takes place under continuous magnetic stir and with the application of heat, around 50°C. At the same time, the oily phase is prepared under the application of heat up to 75°C, in order to manage the complete melting of the oils. The emulsification process takes place, when both phases are completely solubilized and at the same temperature. The addition of the oily phase in to the aqueous takes place under medium stir. The apple extract with the rest of the phase C (as seen in Table 1) is added after the emulsion in under 40°C. Finally, the emulsion

Table 1. Formulation of the Face Cream

Phase	Ingredients	Quantity
(A) Aqueous	Aqua, glycerin, sodium phytate; aqua; alcohol, xanthan gum,	65.25
(B) Oily phase	Potassium palmitoyl hydrolyzed wheat protein (and) glyceryl stearate (and) cetearyl alcohol, caprylic/capric triglyceride, <i>Prunus amygdalus dulcis</i> (almond) oil, cetearyl alcohol, <i>Butyrospermum parkii</i> (Shea) butter, <i>Macadamia ternifolia</i> seed oil, <i>Simmondsia chinensis</i> (Jojoba) seed oil, glyceryl stearate citrate	29.00
(C) Phase C	<i>Malus domestica</i> peel extract, allantoin, D-panthenol, tocopheryl acetate, benzyl alcohol	5.75

is homogenized at 4600 rpm in order to be completely homogenous and light textured.

Evaluation of the antioxidant activity of the extract in the emulsion

The final formulations of the face creams were evaluated with the aim to prove the antioxidant effectiveness of the extract within the product. For comparative reasons, a base cream was prepared, in which no antioxidant was incorporated. First, 1 g of the cream is extracted in absolute ethanol for 10 minutes. The extract is filtered through 0.45 µm pore size filters and the sample is treated like the samples of the extracts as described previously. The same protocol is repeated after 40 days for stability purposes.

Physicochemical tests for the formulation

The formulation of the emulsion was tested through various tests. The organoleptic characteristics of the formulation, color, odor, thickness as well as mechanical integrity, homogeneity, after feel effect, ease of removal and loss on drying. In addition to these, the formulation was tested for its stability through freeze-thaw studies and cycling test. Its stability in different temperatures and rates of humidity was tested in house and in another outsourcing laboratory.

Homogeneity is tested by visual appearance and by touch. Mechanical integrity is tested by centrifugation at 4000 rpm for 30 minutes. After-feel effect is tested by the emolliency, slipperiness and the amount of residue left after the application of fixed amount of cream. Ease of removal, is examined after the washing of the cream from the hand with tap water. Loss on Drying, is determined after evaluating the amount of water that is evaporated after keeping for 2 hours in 105°C in the oven 1 g of the cream. Freeze thaw studies are consisting of 5 complete cycles, where for each cycle, the sample is stored for 8 hours in the freezer and then it is permitted to thaw at room temperature for 16 hours and evaluated for its appearance odor, texture and color.

On the other hand, cycling tests are consisting of 6 cycles and each cycle is consisting of two 24 hours sub-cycles. During the first cycle, the sample is remaining for 24 hours in the oven with a stable temperature of 40°C ± 2 and after it is moved for 24 hours in the cold storage at 4°C. After each cycle, samples are observed for the occurrence of phase separation.

Last, in-house stability testing is performed for 14 weeks in different storage conditions, (1) Room Temperature, (2) at 4°C, (3) at 40°C, (4) Window Storage and (5) In the dark. Samples are assessed each week for their appearance, Odor, texture, Colour, and pH. For the pH measurement, 0.5 g of the cream are diluted in 50 mL of deionized water and pH is measured with a pH meter.

Differential Scanning Calorimetry

For an emulsion system, differential scanning calorimetry (DSC), can be used in order to estimate the freezing and melting temperature of the emulsion, the type of the emulsion and the stability of the system. In our study, we used the following programme in order to determine the properties of the emulsion: it started with an isothermal programme by remaining at 30°C for 10 minutes, then cooling down to -60°C with a rate of 3 K/min and remain there for 5 minutes. Finally, the sample is heated back to 30°C, with a rate of 3 K/min. The experiment is performed using DSC3, Mettler Toledo. The inert gas is N₂, with a rate of 50 ml/min. Previously, the instrument is calibrated with a standard method using Indium as standard material. The software, with which the results are evaluated, is STARe Software.²⁶

Stability testing of the final product

The objective of this study is to ensure that the face cream maintains its intended physical, chemical and microbiological quality and properties, as well as its functionality and aesthetics when stored under specific conditions. When the testing is completed, the provided data can help to determine product stability overtime within its useful life span and verify the compatibility between the container material and the formulation. This study was conducted in an outsourced certified Laboratory, QACS LAB/Quality Assurance and Control Systems LTD (1 Antigonis St., 14451, Athens, Greece, Tel: +30 210 2,934,745, Fax: +30 210 2,934,606, email: info@qacs.gr, web-site: www.qacs-lab.com). The Test Report Number is 19 26 00422 and the study's references are: ICH Q1 A (R2), EC 1223/2009, COLIPA Guide on stability testing of cosmetic products, 2004 and IFSCC Monograph No2 The fundamentals of stability testing.

In order to test the sensorial, physical, chemical and microbiological attributes of the product as well as the preservation system, since these are the parameters susceptible to change during storage and can directly impact the overall quality, safety and performance characteristics of the product, the method is designed accordingly. The action limit is set at a ±20% variation from the initial assay value (time zero) and as a reference product, a sample is stored at room temperature.

The protocol includes the evaluation of the below parameters at specific storage conditions and time intervals:

- Organoleptic: appearance, color, odor and texture.
- Physical-chemical: pH, weight, assay of preservatives.
- Microbiological parameters: microbial count and preservation efficacy test.
- Compatibility between product and container

Conditions

Stability room temperature: the samples are stored at

room temperature (25°C ± 2°C). At the beginning of the stability all parameters are evaluated. The same applies for the conclusion of the study (3 months), where parameters are re-examined.

Stability accelerated (40 °C/75% RH): The samples are placed in a chamber and organoleptic and physicochemical controls are carried out weekly. The same applies for the compatibility study between the container and the formulation. At the conclusion of the study, all the above parameters are re-examined as well as the preservation system (through challenge test, see above).

Stability refrigerator (5°C): The samples are stored in the refrigerator (5°C ± 2°C). Organoleptic and physicochemical controls are carried out weekly. Upon conclusion of the study (3 months) physicochemical parameters are also re-examined.

Stability cycles: The samples are stored in a freezer (-20°C ± 2°C) for eight hours and permitted to thaw at room temperature. The organoleptic and sensorial attributes are evaluated. The same samples are left at room temperature (25 °C ± 2 °C) for 16 hours and then reevaluated. The study is completed after five days, during which a total of 5 freeze – thaw cycles are completed.

Stability dark and window: Samples are kept in a closed cabinet (dark) and nearby a window (diffused light). Organoleptic sensorial attributes, are evaluated weekly. The study is completed within three months.

As reference product, a product was kept in an inert, impermeable container, with which it cannot interact and which fully protects it from the ambient atmosphere.

Challenge test

The challenge tests of cosmetics, which is also mentioned as preservative efficacy test (PET), constitutes vital part of the product information file (PIF) and the cosmetic product safety report (CPSR) uses it as a reference. ISO 29621 states that “every cosmetic manufacturer has to ensure that the product, as purchased, is free from the numbers and types of microorganisms that could affect product quality and consumer health, as well as to ensure that microorganisms introduced during normal product use will not adversely affect the quality or safety of the product”. In other words, this test proves the preservation efficacy of the cosmetic product.

The test consists of challenging the preparation, with a prescribed inoculum of suitable micro-organisms, storing the inoculated preparation at ambient temperature, avoiding sunlight, withdrawing samples from the container at specified intervals of time and counting the organisms in the samples so removed. The preservative properties of the preparation are adequate if, in the conditions of the test, there is a significant fall or no increase, as appropriate, in the number of microorganisms in the inoculated preparation after 2, 7, 14 and 28 days. This study was conducted in at outsourced certified Laboratory

also, QACS LAB/Quality Assurance and Control Systems LTD (1 Antigonis St., 14451, Athens, Greece, Tel: +30 210 2,934,745 Fax: + 30 210 2,934,606 email: info@qacs.gr website: www.qacs-lab.com). The Certificate ID is: 2019-11867/19 01 04484. In Table 2, the parameters of challenge test are de-scribed and in Table 3 the official criteria. More information for these can be found at international cosmetics challenge test standard ISO 11930:2019 or the United States Pharmacopeia standard USP 51.

Certificate of analysis

The final product is examined for the presence of potent allergens through different analytical methods (HPLC, GC-MS). This analysis was conducted in at outsourced certified Laboratory also, QACS LAB/Quality Assurance and Control Systems LTD (1 Antigonis St., 14451, Athens, Greece, Tel: +30 210 2,934,745 Fax: +30 210 2,934,606 email: in-fo@qacs.gr website: www.qacs-lab.com). The Certificate No: 2019-11869/19 10 03961.

Results

Extraction and antioxidant evaluation of the extracts

The results of the antioxidant activity of each extract were evaluated in vitro, based on the DPPH (1,1-Diphenyl-2-picryl-hydrazyl) assay. This is a stable free radical with a characteristic absorption at 517 nm. When a decrease in its absorbance is observed, it is because of the scavenge of it by an electron or a hydrogen donation from an antioxidant source. Each extract of the tree different protocols was tested in different concentrations and all together at a competitive study, where the same concertation of each extract was deter-mined for its scavenging activity. The results are displayed at Table 4 and Figure 1.

Antioxidant activity of the emulsion

The extraction of the antioxidants from the cream showed the presence of antioxidant substances inside the final formulation which were compared to the antioxidants in the same emulsion without the addition of the apple

Table 2. Microbiological test on receipt.

Parameter	Report	Units
Total microbial count	<10	c fu/g
Moulds & yeasts	<10	cfu/g
<i>Pseudomonas aeruginosa</i>	Absence per g	-
<i>Staphylococcus aureus</i>	Absence per g	-
<i>Candida albicans</i>	Absence per g	-

Table 3. European Pharmacopoeia, Criteria A (B), Limits in Log Reduction Units

Time	2 Days	7 Days	14 Days	28 Days
Bacteria	2	3	-(3)	NI
Moulds & yeasts	-	-	2 (1)	NI

Where NI: No Increase.

extract (Figure 2). Moreover, the final formulation proved to maintain stable antioxidants after 40 days.

The antioxidant activity of the face cream incorporating the apple extract is presented in Figure 2, showing a superiority compared to the base cream. At Figure 3, it is presented the antioxidant value of the face cream after 40 days of storing at room temperature.

Differential scanning calorimetry

The advantage of using DSC over other methods, it that it can accurately provide us with information about thermal characterization of physicochemical transformations that are going on in the emulsion, in this case solidification and melting of the droplets. This kind of data, could be further

Table 4. Results of the Percentage (%) of the Scavenging Activity of Each Extract Based on the DPPH Assay

Protocol	Concentration (µg/mL)				
	50	100	200	400	800
Protocol A	25%	22%	21.4%	20.1%	24%
Protocol B1	<0%	<0%	<0%	<0%	<0%
Protocol B2	<0%	<0%	<0%	<0%	<0%
Protocol C	22.8%	42.1%	67.9%	82.1%	82.1%

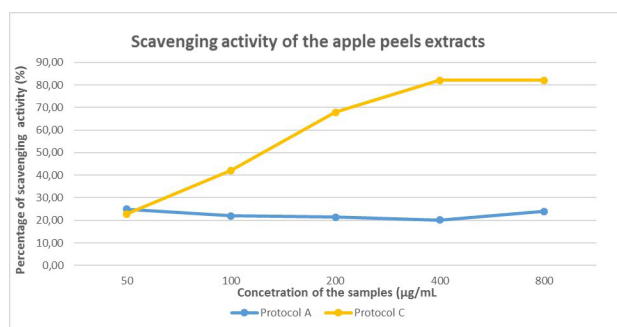


Figure 1. Scavenging Activity of the Apple Peels Extracts. The scavenging activity was evaluated using the DPPH protocol and only extracts from extraction method A and C had results above 0. As the results clearly state, extracts obtained from Protocol C, appear to be more effective towards free radicals.

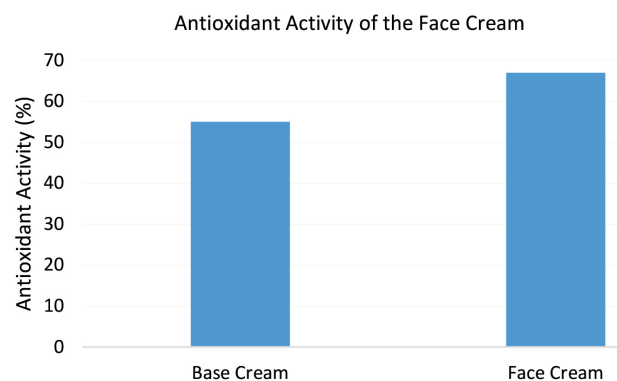


Figure 2. Antioxidant Activity of the Face Cream. As base cream, is described the emulsion without the addition of ingredients with antioxidant value. The creams were extracted in alcohol and assessed based on the DPPH assay.

analyzed in order to evaluate the type of the emulsion, the amount of water, its stability etc. The energy that is absorbed or emitted during each program, is connected to the type of reaction that take place, depending if it is an endothermic or an exothermic. The principle of DSC is the difference in the heat-flux between the reference substance and the sample substance, as a function of temperature, when both substances are subjected to a controlled thermal program. Because heat capacity of each material is one of the main thermal properties of it, heat flux is a vital factor in the experiment, which is connected to the phenomenon of emitting or absorbing of heat.²⁶

What interests us in the case of emulsions, is the freezing and the melting temperature. During the cooling of the sample, the water droplets begin to freeze, which is an exothermic reaction. Freezing temperature, is based on the water concentration the sample. According to the nucleation theory, the volume of the water determines the freezing temperature. Melting, is on the contrary an endothermic reaction which is usually near 0°C, which is practically the temperature of the melting of ice. In Figure 4, the DSC thermograph of the emulsion is presented. Freezing temperature is at -16°C and melting temperature around 0 °C. Here can be stated that the fact that the exothermic freezing peak appears to be extremely sharp at the beginning, is due to the amount of energy that is released in a very short time. Based on the work from Dalmazzone et al,²⁶ the thermograph of the emulsion here is close to the one they propose as an o/w emulsion thermogram. In the case of w/o emulsion, they underline

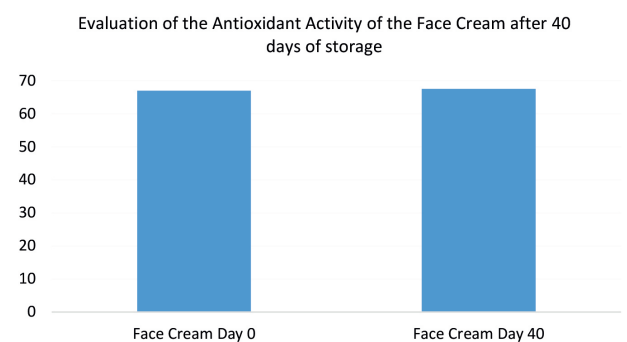


Figure 3. Evaluation of the Antioxidant Activity of the Face Cream After 40 Days of Storage. Storage conditions were 25°, <60% RH. The creams were extracted in alcohol and assessed based on the DPPH assay.

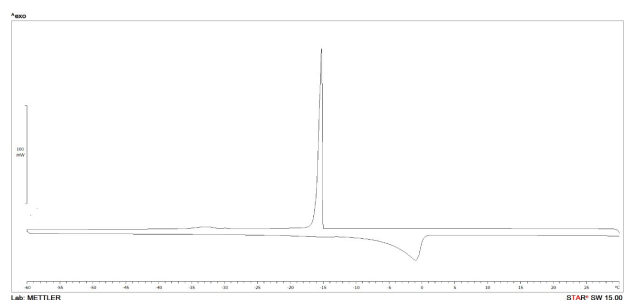


Figure 4. Thermogram of the Face cream, DSC 3 Mettler, Toledo.

that the freezing peak is bell-shaped, rather than sharp. In addition to this, the fact that the peaks of the emulsion thermogram have relatively small droplet size and their peaks describe a rather stable system.

Determination of the flavonoid content

The colorimetric method that was used in this study was proposed by Wolfe et al.³¹ in order to evaluate flavonoid content with some modifications. Quercetin was used as the reference substance (Figure 5). Its standard curve (1–12 µg/mL) was used for the determination of the flavonoids in the samples. The evaluation of the flavonoids in the samples showed that with increasing concentrations of the extract, an increased flavonoids concentration was indicated (Figure 5). Furthermore, based on the solution dilution equation, in all samples tested, an average concentration of 1527,2 µg/mL of flavonoids was observed. The sample solution after the addition of the reagents ended having a pink undertone color. At this point it is worth mentioning that extracts from the other protocols³³ where also evaluated but no significant results were observed, thus they are not presented here.

Determination of the anthocyanin content

The spectroscopic pH differential protocol that was based on the work from Wolfe et al.³¹ showed the presence of anthocyanins in the extract. Specifically, 1 mL of apple peel extracts was added and diluted to a final volume of 10 mL, in two different buffers. The first buffer was KCl/HCl buffer pH 1, and the second was Sodium Acetate Buffer pH 4.5. Apart from the difference in pH, they exhibited different colors after the addition of the extract. Using the equation (2), the amount of the total monomeric anthocyanins is estimated approximately at 13.025 mg/L, since A = 0.078.

Stability testing of the final product

The stability study results judged Appearance, Odor, Texture, Colour and the pH of the products under different conditions, 25°C, 40°C/ 75% RH and at 5°C. Summing up the results, the organoleptic characteristics did not show any kind of change during these 12 weeks compared to the reference product. The pH of the product stored at 25°C varied from 5.58 to 5.44 of the product at 40°C from 5.58 to 5.25 and from the one at 5°C, from 5.58 to 5.46.

Table 5. Results of the Challenge test of the Final Product

Parameter	ATCC	Inoculation	0 time	2 nd day	7 th day	14 th day	28 th day
<i>Pseudomonas aeruginosa</i>	9027	4.8E+05	4.8E+05 4.5E+05	<100	<10	<10	<10
<i>Staphylococcus aureus</i>	6538	5.2E+05	3.1E+05	<100	<10	<10	<10
<i>E. coli</i>	8739	7.8E+05	7.0E+05	<100	<10	<10	<10
<i>Candida albicans</i>	10231	3.8E+05	3.5E+05	<100	<10	<10	<10
<i>Aspergillus brasiliensis</i>	16404	3.0E+05	2.9E+05	2.8E+03	1.2E+03	7.7E+02	<10

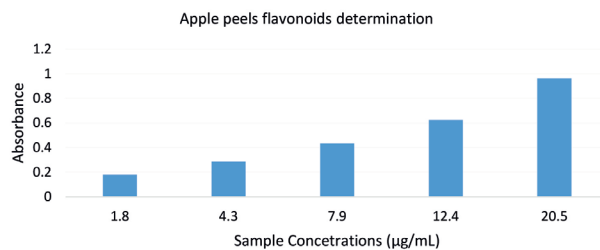


Figure 5. Apple peels Flavonoids Determination.

At the same time the products stored at window and at dark, did not appear to have any significant changes compared to the reference product and their organoleptic properties remained the same. Finally, the stability studies (freeze thaw studies) showed that the product remained stable after each cycle.

Challenge test

The results are indicated below in Table 5. They meet the relevant A-criteria. As the results stated at Table 6 describe, the product contains a powerful preservation system that can significantly decrease the presence of these microorganisms. The study showed that the product meets the criteria of a safe product that can defend against the different types of microorganisms if an unexpected contamination occurs.

Certificate of analysis

The presence of potent allergens is presented in the following table (Table 6). The product does not contain any ingredients that can cause allergies or be a source of skin irritations. The only detectable allergen is benzyl alcohol, which is the preservative that is used in this case. However, its presence is controlled in order to be safe for consumers.

Discussion

Apples (*Malus domestica*), are considered to be the most common fruit. Their impact on the overall wellness through nutrition is known and widely accepted. Specifically, the consumption of them with their peels is said to be even more beneficial since they contain different antioxidant compounds, like polyphenols. In this study, we tried to make extracts using the most common procedures as they are described in literature and evaluate their efficacy.

Table 6. Analysis of the Potent Allergens in the Final Product

Parameters	Method	Result	Units
Amyl cinnamal (CAS No. 122-40-7)	GC-MS	<1	mg/kg
Amylcinnamyl alcohol (CAS No. 101-85-9)	GC-MS	<1	mg/kg
Anise alcohol (CAS No. 105-13-5)	GC-MS	<1	mg/kg
Benzyl alcohol (CAS No. 100-51-6)	HPLC	10002	mg/kg
Benzyl Benzoate (CAS No. 120-51-4)	GC-MS	<1	mg/kg
Benzyl Cinnamate (CAS No. 103-41-3)	GC-MS	<1	mg/kg
Benzyl salicylate (CAS No. 118-58-1)	GC-MS	<1	mg/kg
Cinnamal (CAS No. 104-55-2)	GC-MS	<1	mg/kg
Cinnamyl alcohol (CAS No. 104-54-1)	GC-MS	<1	mg/kg
Citral (CAS No. 5392-40-5)	GC-MS	<1	mg/kg
Citronellol (CAS No. 106-22-9)	GC-MS	<1	mg/kg
Coumarin (CAS No. 91-64-5)	GC-MS	<1	mg/kg
Eugenol (CAS No. 97-53-0)	GC-MS	<1	mg/kg
Farnesol (CAS No. 4602-84-0)	GC-MS	<1	mg/kg
Geraniol (CAS No. 106-24-1)	GC-MS	<1	mg/kg
Hexyl cinnamal (CAS No. 101-86-0)	GC-MS	<1	mg/kg
Hydroxycitronellal (CAS No. 107-75-5)	GC-MS	<1	mg/kg
Isoeugenol (CAS No. 97-54-1)	GC-MS	<1	mg/kg
Butylphenyl methylpropional (CAS No. 80-54-6)	GC-MS	<1	mg/kg
Limonene (CAS No. 5989-27-5)	GC-MS	<1	mg/kg
Linalool (CAS No. 78-70-6)	GC-MS	<1	mg/kg
Hydroxyisohexyl 3- cyclohexene carboxaldehyde (CAS No. 31906-04-4)	GC-MS	<1	mg/kg
Methyl-2-octynoate (CAS No. 111-12-6)	GC-MS	<1	mg/kg
alpha-isomethylionone (CAS No.127-51-5)	GC-MS	<1	mg/kg
<i>Evernia furfuracea</i> extract (CAS No. 90028-67-4)	GC-MS	<1	mg/kg
<i>Evernia prunastri</i> extract (CAS No. 90028-68-55)	GC-MS	<1	mg/kg

The best solvent combination proved to be Acetone 80%. In the same extract, flavonoids and anthocyanins were evaluated. Flavonoids were evaluated to be at an average concentration of 1527.2 µg/mL. Anthocyanins as total monomeric anthocyanins is estimated approximately at 13.025 mg/L. This extract was formulated in a final product with antioxidant claims. The final product proved to have significant antioxidant properties boosted by the addition of the extract. Moreover, the studies showed that it is a stable product even under extreme circumstances,

with adequate preservative efficacy and without potent allergens. Benzyl alcohol is the only allergen appeared in the formula, which is something acceptable and within the limits. Its presence was due to the addition of it as a preservative on the formula. The official database for ingredients (cosIng, the European Commission for Ingredients)³⁴ allow benzyl alcohol at levels until 1%. This ingredient proved to provide a successful preservation system as the challenge test showed. To be more precise, through “feeding” the product with different kind of microorganisms, the product could significantly decrease and even reach low levels of these species, showing that the product can efficiently defend dangers during its storage or use.

Stability Studies presented very pleasant results during different conditions and cycles. Freeze-thaw Studies, extreme conditions, light and air etc. were applied but the emulsion remained stable and both its organoleptic properties and pH did not show any changes. In addition to these results, the DSC thermogram had a typical look of a o/w emulsion.

Conclusion

Free radicals are naturally formed within the human body and often when they are produced they can react with the body in a destructive way. For example, they can interact with some proteins like collagen which is recognized for keeping skin fresh and vigorous and prevent their activity. Their effect may not be visible at first but after a constant exposure, the result is visible and irreparable. The only shield against these radicals, apart from avoiding exposure, are antioxidants. Antioxidants react with free radicals before they manage to cause any further damage.

The application of a face cream is a fundamental part of every woman’s daily skincare routine. Moisturization, hydration, brightness, are a few of the most popular claims that women seek. It is a basic step which can never be neglected. In addition to this, women are seeking for products with unique characteristics, innovative formulas and attractive results. Products with antioxidant ingredients always draw the attention, since like in medicine the same applies for skincare, prevention is the best cure. Nowadays consumers, are accepting an important amount of information over cosmetics and this has raised their awareness. They tend to seek for products with less chemical ingredients more nature-oriented additions. Therefore, the research for new natural ingredients should be encouraged.

This research study showed a simple yet effective way to separate antioxidants from a common natural source, and use them in an everyday product. The scope was to have a complete final product, safe to use based on the mandatory studies of every cosmetic product but with maintaining its antioxidant properties.

Competing Interests

None.

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34. CosIng is the European Commission database for information on cosmetic substances and ingredients. <https://ec.europa.eu/growth/tools-databases/cosing/>.

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