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Corresponding Author: Dr H. Dominguez,

Corresponding Author's Institution:

First Author: H. Dominguez

Order of Authors: H. Dominguez; Elena M Balboa; Maria L Soto; Danielle R Nogueira; Noelia Gonzalez-López; Enma Conde, PhD; Andres Moure; Maria P Vinardell, PhD; Montserrat Mitjans, PhD

Abstract: The performance of natural extracts obtained from underutilized and residual vegetal and macroalgal biomass processed with food-grade green solvents was compared with that of commercial antioxidants. Selected extracts were obtained from two terrestrial sources: winery byproducts concentrate (WBC) and chestnut burs hydrothermally fractionated extract (CBAE), and from two underutilized seaweeds: Sargassum muticum extracts, either extracted with ethanol (SmEE) or after alginate extraction and hydrothermal fractionation (SmAE) and from Ulva lactuca processed by mild acid extraction and membrane concentration (UlAE). These extracts showed in vitro antioxidant properties comparable to commercial antioxidants and were safe for topical use based on the absence of skin-irritant effects at 0.1% on reconstructed human tissues. The stability of several cosmetic model emulsions was assessed during accelerated oxidation assays. The incorporation of natural extracts produced from renewable underutilized resources at 0.4-0.5% in an oil in water emulsion reduced lipid oxidation during storage.

Grupo de Farmacología-Toxicología, Universidad de La Habana, Ciudad de La Habana, Cuba
ana@fbio.uh.cu

Natural extracts from underutilized biomass processed with food-grade green solvents performed similarly to commercial antioxidants

These extracts were safe for topical use

The incorporation of these extracts at 0.4-0.5% in oil-in-water emulsions reduced lipid oxidation during storage.

Potential of antioxidant extracts produced by aqueous processing of renewable resources for the formulation of cosmetics

Elena M. Balboa[1,2], Maria Luisa Soto[1], Daniele R. Nogueira[3], Noelia González-López[1,2], Enma Conde[1,2], Andrés Moure[1,2], María Pilar Vinardell[3], Montserrat Mitjans[3], Herminia Domínguez[1,2*]

[1] Dep. Enxeñería Química, Universidade de Vigo (Campus Ourense), Edificio Politécnico. As Lagoas s/n, 32004, Ourense. Spain

[2] CITI-Universidade de Vigo, Parque Tecnolóxico de Galicia, Rúa Galicia nº 2, 32900 Ourense, Spain

[3] Dep. Fisiologia, Facultat de Farmàcia, Universitat de Barcelona, Av. Joan XXIII s/n, 08028 Barcelona, Spain

ABSTRACT

The performance of natural extracts obtained from underutilized and residual vegetal and macroalgal biomass processed with food-grade green solvents was compared with that of commercial antioxidants. Selected extracts were obtained from two terrestrial sources: winery byproducts concentrate (WBC) and chestnut burs hydrothermally fractionated extract (CBAE), and from two underutilized seaweeds: *Sargassum muticum* extracts, either extracted with ethanol (SmEE) or after alginate extraction and hydrothermal fractionation (SmAE) and from *Ulva lactuca* processed by mild acid extraction and membrane concentration (UlAE). These extracts showed *in vitro* antioxidant properties comparable to commercial antioxidants and were safe for topical use based on the absence of skin-irritant effects at 0.1% on reconstructed human tissues. The stability of several cosmetic model emulsions was assessed during accelerated oxidation assays. The incorporation of natural extracts produced from renewable underutilized resources at 0.4-0.5% in a oil-in-water emulsions reduced lipid oxidation during storage.

Keywords: winery byproducts, chestnut burs, *Sargassum muticum*, *Ulva lactuca*, autohydrolysis, oxidative stability

*Corresponding author

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1. INTRODUCTION

The growing demand for natural additives is being incentivized by the restricted use of synthetic antioxidants to prevent lipid oxidation in food and cosmetic products (Andreassi and Andreassi. 2004). Agro-industrial by-products are cheap, abundant and sustainable resources, which contain compounds with antioxidant, cytotoxic and antimicrobial activities that could be proposed as a natural preservative in cosmetic products (Vinardell *et al.*, 2008; Rodrigues *et al.*, 2013). In addition, formulations enriched in antioxidants administered topically by cosmetics or by diet supplements, could exert an antioxidant/protective effect in skin by decreasing the oxidative stress (Morganti *et al.*, 2002), which is one of the major mechanisms for skin aging.

An important concern related to the extraction of natural ingredients for cosmeceutical uses is the desired limitation of toxic solvents (Chaudhari *et al.*, 2011). Water is an abundant solvent, but not suited to extract non polar compounds. However, hot pressurized water under subcritical conditions possesses dielectric properties similar to some apolar solvents. One of the available technologies operating with subcritical water is autohydrolysis or hydrothermal treatment at temperatures in the range 150 to 250 °C. This autocatalyzed reaction has been proposed for the environmentally friendly fractionation of vegetal biomass (Conde *et al.*, 2011).

Natural phenolics from terrestrial sources, including benzoic acids, cinnamic acids and flavonoids, possess antioxidant, cardioprotective, neuroprotective, anticancer, anti-inflammation, antiaging and antimicrobial properties (Boudet, 2007; Vinardell *et al.*, 2008). Phlorotannins are phenolic compounds exclusive of marine seaweeds, but these organisms, particularly brown algae, also contain a variety of components with antioxidant action (such as polyunsaturated fatty acids, proteins, pigments, vitamins, polysaccharides and carotenoids). The phlorotannins and algal polysaccharides exhibit anticoagulant, antiviral, antioxidative, anticancer, antiinflammatory and immunomodulatory actions, and could have potential for the development of nutraceutical, pharmaceutical and cosmeceutical products (Batista González *et al.*, 2009; Balboa *et al.*, 2013; Thomas and Kim, 2013). Valorization and utilization of the constituents of the brown macroalga *Sargassum muticum* was proposed, since the solvent extracts (Kim *et al.*, 2007) and the soluble fractions obtained after autohydrolysis (González-López *et al.*, 2012) were active *in vitro* antioxidants. The green alga *Ulva lactuca* does not possess an important phenolic fraction, but contains other components such as polysaccharides and steroids showing bioactive properties (Lahaye and Robic, 2007).

The aim of this work was to evaluate the cosmetic potential of extracts produced from biorenewable underutilized sources using green solvents: two concentrated and refined fractions from terrestrial

sources and three crude extracts from seaweeds. The absence of skin irritating properties and the protection against oxidation of model cosmetic products were assessed.

2. MATERIALS AND METHODS

Extracts

A winery byproducts concentrate (WBC) was prepared from distillery wastes produced in December 2009 (Cooperativa Vitivinícola Ribeiro, Ourense, Spain). The liquid stream separated by pressing was centrifuged to remove suspended solids and phenolic compounds were recovered and concentrated to obtain a light colored powder extract (Díaz *et al*., 2012).

Chestnut (*Castanea sativa*) burs, collected in Autumm 2006 in Ribeira Sacra (Ourense, Spain), were processed by non isothermal autohydrolysis (using water as the only reagent). The liquid phase was further refined by extraction with ethyl acetate, washing with ethanol/water solutions, adsorption onto a non-ionic polymeric resin (Sepabeads SP700, Resindion S.R.L., Mitsubishi Chemical Corp.) and elution with ethanol. The dark brownish powder extract (CBAE) was prepared by freeze-drying (Conde *et al*., 2011).

Sargassum muticum, collected in Mourisca Beach, Alcabre (Pontevedra, Spain) in Summer 2010, was separated from other species, washed with tap water, oven dried at 50 °C, milled and stored at room temperature in sealed plastic bags until use. Ground *S. muticum* was further extracted with 96% ethanol. After filtration to separate solids and vacuum evaporation of the liquid phase to remove the solvent, a dark green solvent extract named SmEE was obtained. The ground *S. muticum* biomass was also processed by conventional technology for the solubilization of alginate, with sequential extractions using 1% formaldehyde, 0.2 N sulphuric acid, 1% sodium carbonate and intermediate washings with tap water. Alginate was recovered from the sodium carbonate soluble fraction and the solid residue remaining after alginate extraction was treated with water, at a liquid:solid ratio of 30:1 g:g, in a batch reactor (Parr Instr. Co., Moline, IL) under non-isothermal conditions until the equipment temperature reached 190 °C. The liquid phase or autohydrolysis liquors were recovered by filtration and freeze-dried, this dark brown powder product was named SmAE and contained both phlorotannins and fucoidan fractions (González-López *et al*., 2012).

Ulva lactuca, removed during cleaning of shellfish banks in February 2011 in Vilagarcía de Arousa (Pontevedra, Spain), was manually separated from other species, washed with tap water, oven dried at 50 ºC, milled to a particle size of less than 1.0 mm and stored at room temperature before processing. Algal biomass was subjected to an acid hydrolysis with 1.25% H_2SO_4 in glass bottles in an autoclave using a liquid:solid ratio of 60:1 at 120 ºC during 1 hour. The liquid obtained after filtration was neutralized adding $CaCO_3$. A second filtration to remove $CaCO_3$ was necessary. This liquid phase was

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concentrated thrice by a Prep/Scale spiral membrane (Millipore, TFF6, 1 kDa, 0.54 m^2 filtration area, made of regenerated cellulose) operating with 200L/h at 4 bar and 22 ºC. The retentate was freeze-dried to obtain a slightly yellow powder extract (UlAE) and stored until use.

Cosmetic model products

Two creams, avocado cream and suncream and two cosmetic oils, massage oil and shower oil, were chosen for the experiments. The extracts were added as antioxidants in the cosmetics preparations.

Avocado cream (AC) was formulated with the following components (g): avocado oil (25), sorbitan monolaurate (5), water (100) and antioxidant (0.4). All the ingredients were mixed, homogenized with sonication and neutralized with triethanolamine (if the mixture had acid pH), to form the emulsion. The extracts tested in this cream were: tocopherol (T), *Sargassum muticum* ethanol extract (SmEE) and autohydrolysis extract (SmAE), chestnut burs autohydrolysis extract (CBAE) and winery byproducts concentrate (WBC).

Sun cream (SC) was prepared with an *oil phase* containing (g): cream basis (o/w) (18), dimethicone 350 (6), avocado oil (3), sunscreen (8), titanium dioxide (18), antioxidant (0.75), Fenonip (0.35) and a w*ater phase* containing propylene (6), carbopol ultrez 10 (1.5), triethanolamine (1.5), demineralized water (80). The melted oils were mixed in a water bath, until the water temperature achieved 70ºC. When the mixture raised 40 ºC, 0.45 mL of bergamot oil and 3 mL of tetramer cyclomethicone were added. The carbopol and the propylene were separately added to the water, sonicated and neutralized with triethanolamine to form a gel. The oily phase was mixed with the aqueous one with constant stirring. The compounds and extracts tested in this cream were T, tea extract (TE) (Guinama, Valencia, Spain), commercial vine extract (VE, Guinama, Valencia, Spain), WBC and *Ulva lactuca* powder extract (UlAE).

Massage oil (MO) was composed of the following ingredients (g): glyceryl tricapryl-caprate (42.5), octyldodecanol (25.5), isopropyl myristate (22), almond oil (6), menthol (1.5), camphor (1.5) and antioxidant (0.5). The oil was prepared by adding the camphor and the menthol to the almond oil, the mixture was stirred with a spatula and after the addition of the other components, it was sonicated for 4 minutes. The extracts tested in this oil were T, TE, SmEE and *Fucus* extract (FE) (Guinama, Valencia, Spain).

Shower oil (SO) was formulated by mixing and homogenizing the following components (g) in the proposed order: octyldodecanol (35), isopropyl myristate (20), antioxidant (0.5), essence of roses (0.3), glyceryl tricapryl-caprate (44). The extracts tested in this oil were: T, TE, SmEE, and FE.

The antioxidant extracts were added to the oil dissolved in ethanol. Control samples without any extract added were also prepared and analyzed.

Analytical methods

Total phenolic content was colorimetrically determined using the Folin-Ciocalteu reagent (Sigma-Aldrich, St. Louis, USA) and expressed as gallic acid (Sigma-Aldrich, St. Louis, USA) equivalents. All analyses were performed at least in triplicate and are reported on a dry matter basis. Ash content was gravimetrically determined. The monomeric sugars: glucose, xylose, mannose, fucose and galactose, estimated from the concentrations of monosaccharides in samples previously hydrolyzed with 4% sulfuric acid at 121 °C for 20 min, were assayed by HPLC in a 1100 series Hewlett-Packard chromatograph fitted with a RI detector and a 300×7.8 mm Aminex HPX-87H column (BioRad, Hercules, CA) operating at 60 °C (mobile phase: 0.003 M H_2SO_4, flow rate: 0.6 mL/min).

Antioxidant activity

Ferric reducing antioxidant power (FRAP), reducing power and the scavenging capacity of DPPH (**α,α-Diphenyl-β-picrylhydrazyl) and ABTS** (ABTS$^{\bullet+}$, 2,2'-azinobis (3-ethyl-benzothiazoline-6-sulfonate)) was measured as by Conde *et al*. (2011). EC_{50} was determined as the concentration causing a 50% inhibition of the radical.

β-carotene oxidative bleaching in a β-carotene/linoleic acid emulsion was measured as reported previously (Miller, 1971). The antioxidant activity was measured by the Antioxidant Activity Coefficient (AAC), calculated as the quotient (Absorbance of extract$_{120 \text{ min}}$ - Absorbance of extract$_{120 \text{ min}}$)·1000/(Absorbance of control$_{0 \text{ min}}$ – Absorbance of control$_{120 \text{ min}}$).

ORAC-FL assay. The fluorescence decay of a mixture of antioxidant, fluorescein and AAPH (2,2´-Azobis (2-methylpropionamidine) dihydrochloride) in relation to a blank, following the method described by Conde *et al*. (2011) was recorded and the results were expressed as Trolox equivalents.

Hemolysis induced by APPH. Blood samples were obtained from rats, following the ethical guidelines of the University of Barcelona, and were collected in tubes containing EDTA as anticoagulant. Red blood cells (RBC) were separated from plasma and buffy coat by centrifugation at 1000 g for 10 min. Hemolysis of RBC mediated by AAPH was measured using a modification of the method described previously (Ugartondo *et al*., 2008). The tested product was added to the RBC suspension in the presence of 100 mM AAPH at 37 ºC for 2.5 h. The IC_{50} of the hemolysis induced by AAPH was determined, as the concentration required for a 50% inhibition.

Erythrocyte lipid peroxidation. The level of malondialdehyde (MDA), a secondary product of lipid oxidation, was determined by measuring thiobarbituric acid reactive substances (TBARS) (Ugartondo *et al*., 2009). This method is based on the extraction of MDA from erythrocyte suspension by

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trichloroacetic acid (TCA) solution and the next reaction of this MDA with thiobarbituric acid (TBA), giving a pink colored complex with maximum absorption at 532 nm (TBAR substances). Lipid peroxidation was induced by incubating erythrocytes with 20 mM H_2O_2 alone or with different compound concentrations for 90 minutes at 37 °C. Following incubation, RBC suspension was mixed with 1 mL of trichloroacetic acid solution 20 % w/v (TCA) to remove potentially interfering substances (Srour *et al*., 2000). Samples were then centrifuged and 1 mL of supernatant was mixed with 1 mL of 1% of 2-thiobarbituric acid (TBA). Finally, samples were heated at 90 ºC for 50 minutes, cooled fast, centrifuged and the absorbance of the supernatant was measured at 532 nm and 600 nm. The degree of lipid peroxidation was expressed as nmol/mL of MDA inferred from the standard curve from tetramethoxypropane. In addition, IC_{50} values were calculated as the antioxidant concentration required for the inhibition of 50% TBAR formation.

In vitro skin irritation

The reconstructed human skin tissue is based on determining cell viability, and cytokine release (interleukin-1α, IL-1α). The reconstructed skin inserts Episkin SNC (Lyon, France) were transferred into 12 wells plates containing 2 mL of maintenance medium and incubated (37 °C, 5% CO_2, >95% humidity). After 24 h, the second column of each plate was filled with maintenance medium. The tested product (10 µL) was dissolved (1%) in distilled water and contacted with each epidermis sample during 15 min. PBS was used as negative control and an SDS solution in distilled water as a positive control. The epidermis samples exposed to the tested products were washed with sterile PBS and incubated in the maintenance medium. After 42 h, the medium was collected in Eppendorfs and frozen at -20 ºC for determining IL1-α (Diaclone ELISA kit, UK). Cell viability was determined by the MTT assay. Tissues were transferred to 2 mL wells containing 0.3 mg/mL of MTT solution and incubated for 3 h under the above conditions. The epidermis tissues were soaked with acidic isopropanol (0.5 mL/tube) to extract the intracellular formazan and these tubes were incubated for 4 h in the dark with periodic vortexing. After incubation the tubes were centrifuged, a duplicate of 200 µL was transferred to a 96-well flat bottom microtitre plate and absorbance at 550 nm was read using acidified isopropanol as blank. Viability was calculated respect to the value (100 %) of the negative control.

Oxidation experiments

Cosmetic emulsions were prepared in triplicate, stored in 25 mL capped glass bottles and placed in the dark in an oven at 50 ºC during a period of 34 days. The bottles were periodically removed from the oven to take samples for peroxide value and p-anisidine determination.

The **peroxide value (PV)** was spectrophotometrically determined according to the method reported by Díaz *et al*. (2003). The oil/emulsion samples (0.3 mL) were added to 3:2 v/v isooctane/2-propanol (1.5

mL) and mixed on a vortex during 10 s three times. After centrifugation for 2 min at 1000 x g, the upper layer (0.2 mL) was used for peroxide quantification using a cumene hydroperoxide standard curve. The **p-anisidine (PA)** value was spectrophotometrically determined (AOCS Official Methods no. cd 18-90). The TOTOX value was used to estimate the oxidative deterioration of lipids. TOTOX value is defined as the sum of both values (peroxide and anisidine); TOTOX = 2 PV + PA.

3. RESULTS AND DISCUSSION

The characterization of the chemical, physical and antioxidant properties of the selected extracts is shown in **Table 1**. The extracts were produced from renewable residual or underutilized sources, collected in the period of maximal abundance and processed by previously optimized technologies. The extracts from winery byproducts concentrate (WBC) and from chestnut burs autohydrolysates (CBAE) were richer in phenolic compounds, whereas those from seaweeds showed a lower content. The major compounds in WBC are monomeric phenolic compounds (gallic acid, catechin, epicatechin, and quercetin) as well as oligomeric and polymeric polyphenols (Díaz *et al*., 2012). The major identified compounds of CBAE were phenolic acids (gallic, 4-hydroxybenzoic, vanillic, syringic, p-coumaric, ferulic) and some flavonoids (rutin, quercetin and apigenin) (Conde *et al*., 2011). The antioxidant activity of algal extracts has also been ascribed to phenolic compounds, accompanying components, and synergism among them. In the genus *Sargassum* a diversity of metabolites, including plastoquinones, chromanols, chromenes, steroids, phenolics, sulfated polysaccharides … have been reported as responsible for antioxidant activity (Ayyad *et al*., 2011). The phenolic content in the SmEE was in the range or lower than those reported for other solvent extracts and fractions from *Sargassum* sp (Ye *et al*., 2009; Kim *et al*., 2007), containing phlorotannin dimers and trimers. Among the compounds identified in *S. muticum* are phloroglucinol, bifuhalol, trifuhalol A, trifuhalol B (Glombitza *et al*., 1978), also the carotenoid fucoxanthin was found in the SmEE (unpublished data). In the products resulting from autohydrolysis of *Sargassum* (SmAE) and mild acid of Ulva (UlAE), also sulfated fucoidans and ulvans, respectively were present (unpublished data). Total sugars of SmAE accounted for 15.2% wt of the extract, and the mass percent of the constituent sugars was Fuc:Xyl:Glu:Gal:Man (40.5:15.0:17.2:24.3:2.9 %wt) (unpublished data) The highest ash content was present in the crude extracts produced by autohydrolysis and acid hydrolysis of seaweeds.

In vitro chemical antioxidant assays

The radical scavenging properties have been characterized using universally accepted assays. The DPPH assay is a simple, easy and accurate method with regard to measuring the antioxidant capacity of natural extracts and abundant literature for comparative purposes is available. For the extracts from terrestrial sources, the most active DPPH radical scavenger extracts was that with higher phenolic

content, with activity comparable to synthetic antioxidants. However, despite the low phenolic content of SmEE, this extract showed DPPH radical scavenging activity, as reported for *Sargassum muticum* (Kim *et al*., 2007), other crude extracts from *Sargassum* sp and purified phlorotannins (Ye *et al*., 2009; Balboa *et al*., 2013). The TEAC value of algal extracts was considerably lower than those presented by benzoic acid and cinnamic acid rich extracts. However, other authors reported important ABTS radical scavenging capacity activity of solvent extracts from algae (Ayyad *et al*., 2011; Balboa *et al*., 2013). The reducing power was high both for the phenolic rich extracts and for fucoidan rich extracts; the reducing capacity of crude polysaccharides and fucoidan from brown algae is well known (Rupérez *et al*., 2002; Chattopadhyay *et al*., 2010). The activity in emulsion was higher for the extracts with higher phenolic content and lower for the algal extracts. The CBAE showed AAC values comparable to that of BHA at a similar concentration.

Antioxidant activity in cell systems

Because of their susceptibility to peroxidation, rat red blood cells have been used as a model to investigate oxidative damage in biomembranes. The most efficient inhibitor, showing the lowest concentration causing 50% inhibition of hemolysis induced by APPH, IC_{50} value, was the refined extract from autohydrolysis of chestnut burs (IC_{50}= 48.2 µg/mL), followed by the winery byproducts concentrate (IC_{50}= 123.2 µg/mL), and by the autohydrolysis extract from *Sargassum muticum* (IC_{50}= 160.5 µg/mL). The ethanolic extracts from *S. muticum* and the aqueous extract from *Ulva* did not show activity against this radical. The activity of *S. muticum* autohydrolysis liquors could be enhanced varying either the hydrolysis final temperature or the pretreatment of the sample. More active extracts were obtained from autohydrolysis at 220 °C of the alginate exhausted alga (IC_{50}= 109.7 ± 19.6 µg/mL), from autohydrolysis at 190 °C of the whole alga (IC_{50}= 130.9 ± 18.8 µg/mL) and from autohydrolysis at 220 °C from the whole alga (IC_{50}= 38.7 ± 6.0 µg/mL) (data not shown). These values were comparable to that attained for (-)epicatechin (34.83 mg/L).

The *in vitro* inhibition of lipid peroxidation on erythrocytes was evaluated by malondialdehyde (MDA), generated as secondary product of the H_2O_2 induced lipid peroxidation, and was measured as thiobarbituric acid reactive substances (TBARS). The reactive species used in this assays is representative for skin oxidative stress processes (Andreassi and Andreassi, 2004; Polefka *et al*., 2012). The IC_{50} values were 200 µg/mL for the winery byproducts concentrated extract, 1000 for extracts from hydrothermal treatments and 5000 for ethanolic extracts from *S. muticum*. A low protection against H_2O_2 lipid peroxidation was observed. The differences in the antioxidant activity in these two methods could be explained by the different hemolytic mechanism used by this oxidant compared to AAPH, this later causing both lipid and protein oxidation.

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Irritability tests

Since the endogenous antioxidant concentration decreases from the outermost to the deeper layers of the skin, natural antioxidants can be applied topically to protect skin from oxidative stress induced by UV radiation, although not all antioxidants have similar affinity for different layers of the skin (Abla and Banga, 2013). In order to confirm the potential of the extracts for topical use, irritability assays with the Episkin test were performed. Data for the cell viability and the cytokine (interleukin-1α, IL-1α) release from *in vitro* assays with reconstituted human epidermis are shown in **Table 2**. The extracts produced during the autohydrolysis stage and the ethanolic extract from the selected materials are not skin irritants for topical use in cosmetic formulations at 0.1%. This potential use is also confirmed by the low release of IL-1α (in the range of negative control). In the view of these results, the extracts are good candidates to take into account to be used in cosmetic formulations.

Antioxidant activity in model emulsions and oils

Antioxidants are often needed to protect against lipid oxidation, which would cause rancidity and undesirable changes in texture, appearance, and quality of cosmetic products. Due to the high susceptibility of emulsified lipids to oxidation, combination of antioxidative techniques could be effective in retarding lipid oxidation and improving the shelf-life, utilization and quality of emulsion systems (Waraho *et al*., 2011). Polarity cannot be used to predict the activity of an antioxidant to inhibit lipid oxidation in emulsions, since other factors such as antioxidant concentration, reactivity, partitioning effects, interactions with other components, and environmental conditions such as pH, ionic strength and temperature, are important. In real systems, antioxidants can interact with other compounds either increasing or decreasing their reactivity. The free radical scavenging action of the selected extracts and their protective effect against oxidation in emulsion could make them candidate for use as natural antioxidants in cosmetic products. To verify its efficiency in protection of cosmetic model products, they were added to replace synthetic or natural commercial antioxidants and then subjected to an accelerated oxidation test. Visual examination of preparations revealed uniformity and homogeneity on colour and phases emulsions. Colour of these products was slightly modified: clear brown-yellow for UlAE, white for TE, brownish for CBAE and light green for SmEE.

When molecular oxygen and unsaturated fatty acids are combined in the presence of heat, light or catalysts hydroperoxides are formed. These compounds are reactive and form new oxidizing products, or secondary oxidation products (aldehydes, ketones, hydrocarbons, and alcohols). Oxidative stability can be assessed by analyzing primary and secondary oxidation products (measured by the peroxide and anisidine values, respectively). The PV quantifies the amount of hydroperoxides, a value which is affected by both formation and decomposition reactions. Hydroperoxides are primary oxidation components that have a lower half-life than secondary oxidation. The anisidine value is a measurement

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of the production of aldehydes during oxidation of fats or oils. The Totox value, an evaluation of both primary and secondary oxidation products, is practical for estimating the extent of oxidative deterioration of lipids. The TOTOX values were used to measure the overall oxidative stability after 35 days of storage and the values recorded for avocado cream and for massage oil and shower oil are shown in **Figure 1**.

In avocado cream, an oil-in-water product, the TOTOX value was mainly related to the PV. In SmEE suplemented creams, this value was lower and decreased from 19 to 34 days. Probably the PV formed during oxidation decompose to secondary oxidation products. A slightly different behaviour was observed for the two *Sargassum* extracts, despite their compositions differs and different mechanism could be involved in the protection of oxidation. The aqueous algal extracts contain saccharidic, proteic and phenolic fractions. In O/W emulsions, proteins and some amino acids also inhibit lipid oxidation by free radical scavenging (Park *et al*., 2012), polysaccharides inhibit lipid oxidation in emulsions by free radical scavenging, transition metal binding, and viscosity enhancement, and chelating agents by decreasing metal reactivity or by partitioning the metal away from the lipid. The use of tocopherol did not significantly improve the stability of the creams, whereas the extracts inhibited lipid oxidation more effectively when they contained higher phenolic concentrations (WBC and CBAE). The prooxidant effect of tocopherol has been previously reported during primary and secondary oxidation in structured lipid based emulsions (Osborn-Barnes and Akoh, 2003), and ascribed to the ability of this molecule to donate a hydrogen atom to a peroxyl radical and becoming a tocopheroxyl radical. Also Winter collected *Laminaria digitata* extracts showed prooxidant action on the methyl linoleate oxidation at 60 °C (Le Tutour *et al*., 1998).

Oxidation experiments confirmed that all sunscream (data not shown) formulations were very stable during a period of 34 days at 50 ºC. PV values were in the range 0.15-0.45 mM cumene hydroperoxide and secondary oxidation was not reached. In the products formulated with *Ulva lactuca* extract, the pH was corrected by addition of triethanolamine to avoid destabilizating the emulsion. With storage time, the different sunscream preparations tended to similar values of absorbance at 510 nm, with values in the range 0.35-0.6 after 25 days (**Figure 2**).

Massage oil is more sensitive to oxidation, thus the PVs were relatively low compared to the pA, showing that the hydroperoxides were decomposed into secondary oxidation products. The increase in the p-anisidine values was very apparent, since the less stable primary oxidation products were decomposed under the tested conditions. The control antioxidant, tocopherol, protected only slightly and could not avoid oxidation of the oil. Despite the algal extracts provided additional protection with regard to tocopherol, oxidation occurred under the studied conditions.

In the shower oil the PVs values were low compared to the PA values. The use of TE extract improved the stability of the oil more efficiently (95.28 %) at 34 days of storage, followed by FE, SmEE and T.

After 34 days of storage, SmEE extract inhibited lipid oxidation more effectively in avocado cream (93.96%) than in massage oil (13.90%) and shower oil (58.58%). Tocopherol was more effective in inhibiting lipid oxidation in shower oil and massage (57.33 and 5.21%, respectively), but did not show ability to protect avocado cream from oxidation. SmEE, SmAE, CBAE and WBC were very effective in the oil-in-water emulsion (avocado cream), showing inhibition percentage values of 93.96, 84.37, 97.64 and 97.59 %, respectively. This finding confirmed the results obtained previously in the assay of oxidative bleaching of β-carotene in emulsion.

It would be expected that antioxidant substances and natural extracts can be used to offer complementary protection. Since the active compounds of the extracts were benzoic and cinnamic acids, flavonoids and phlorotannins either naturally present in the raw materials, or released by depolymerization during autohydrolysis and the algal extracts also contained crude fucoidan and ulvan fractions, it could be expected that the cosmetic products formulated with these extracts could have additional biological properties.

Conclusions

Selected extracts obtained from natural sources of low cost using environmentally benign processes and non toxic solvents (water, ethanol) showed *in vitro* antioxidant activity, measured as radical scavenging activity, reducing power and protection of oxidation in a β-carotene-linoleic acid emulsion. The extracts were non irritant at 0.1% and their ability to protect lipid oxidation of selected model cosmetic oil rich products stored at 50 ℃ for 34 days was confirmed in relation to other commercial extracts from natural sources. Phenolic extracts (SmEE, SmAE,, CBAE, WBC) inhibited the lipid oxidation in the range 84.37-97.64% at 34 days of storage in avocado cream. Based on these results, the extracts could be proposed for cosmeceutical preparations, which are gaining popularity due to their non-toxic character and strong antioxidant activity.

Acknowledgements

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Table 1. Characterization of the extracts obtained from renewable underutilized sources: winery byproducts concentrate (WBC), chestnut bur autohydrolysis extract (CBAE), *Sargassum muticum* ethanolic extracts (SmEE), *Sargassum muticum* autohydrolysis extract (SmAE) and *Ulva lactuca* acidic extract (UlAE).

Physicochemical properties	Extract				
	WBC	**CBAE**	**SmEE**	**SmAE**	**UlAE**
Composition (g/100 g extract)					
Phenolic content (gallic acid eq.)	45.0	56.0	1.0	7.5	1.8
Total C	45.95	45.88	34.80	39.73	19.95
Sacharidic fraction = total sugars	17.84	1.74	10.20	15.20	2.62
Glu (% of saccharidic fraction)	59.7	45.9	-	17.2	18.7
Ara (% of saccharidic fraction)	7.7	-	-	-	-
Fuc (% of saccharidic fraction)	-	-	50.9	40.5	-
Ash	6.17	-	-	33.40	38.69
Color	fair yellowish-brownish-gray	dark brown	dark green	dark brown	light yellow
***In vitro* antioxidant properties**					
DPPH, IC_{50} (mg/mL)	0.358	0.297	0.249	0.720	-
TEAC (g Trolox/g extract)	1.73	1.13	0.05	-	0.028
FRAP (μM $FeSO_4 \cdot 7\ H_2O$)	549.02[a]	574.59[a]	0.72[d]	869.90[c]	0.01[e]
FRAP (mM ascorbic acid)	0.26[a]	0.28[a]	0.48[d]	0.57[c]	0.06[e]
Reducing power (mM ascorbic acid)	0.30[a]	0.24[a]	0.17[f]	2.33[c]	0.18[e]
β-carotene (AAC)	513.73[b]	769.04[b]	311[f]	499[c]	115[e]
Reference	Díaz *et al.* (2012)	Conde *et al.*, (2011)	Unpublished data	González-López *et al.* (2012)	Unpublished data
AAPH, IC_{50} (μg/mL)	123.2 ± 7.6	48.2 ± 10.1	1000 ± 170.0	160.5 ± 14.7	2050.8 ± 302.0
TBARS (mg/L)	200	1.000	5.000	1.000	1.000
Protection (%)	-	-	-	60%	-

Glu: Glucose; Ara: Arabinose; Fuc: Fucose
DPPH: α,α-Diphenyl-β-picrylhydrazyl; $IC_{50,\ BHT}$ = 2.79 mg/mL; $IC_{50,\ BHA}$ = 0.22 mg/mL; $IC_{50,\ Ascorbic\ acid}$ = 0.12 mg/mL
TEAC: Trolox Equivalent Antioxidant Capacity
AAC: Antioxidant Activity Coefficient: AAC_{BHT}= 980.47 ± 2.17; AAC_{BHA}= 886.93 ± 9.98
AAPH: Concentration inhibiting 50% of the hemolysis induced by APPH; $IC_{50,\ Epicatechin}$ = 119.82 μg/mL
[a] 100 ppm extract; [b] 1000 ppm extract; [c] 590 ppm; [d] 10.9g/L; [e] 10g/L; [f] 1.09g/L

Table 2. Cell viability and interleukin-1α (IL-1α) release during *in vitro* studies with the autohydrolysis and ethanol soluble fraction of the studied extracts on reconstituted human epidermis.
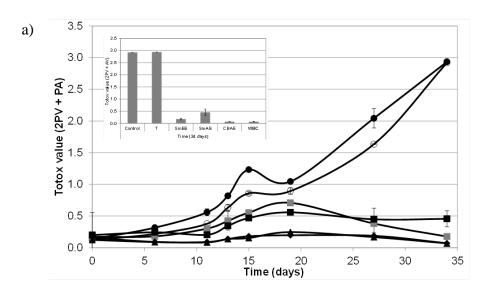
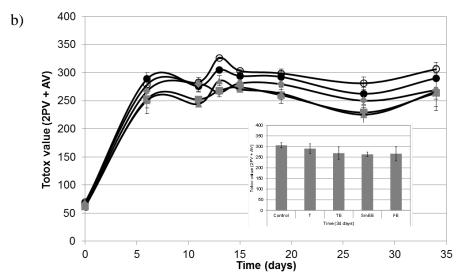| Extract	Optical density	Viability (%)	IL-1α (pg/mL)
Negative control (PBS)	0.77 ± 0.04	100	4.1 ± 2.4
Possitive control (SDS)	0.19 ± 0.08	27.20 ± 12.04	598.4 ± 35.0
SMAE (*S. muticum* autohydrolysis extract)	0.64 ± 0.15	83.01 ± 19.57	0.7 ± 2.3
SMEE (*S. muticum* ethanolic extract)	0.49 ± 0.21	79.44 ± 16.27	3.8 ± 1.6
ULAE *(U. lactuca* acid hydrolysis extract)	0.65 ± 0.18	78.17 ± 26.70	2.4 ± 3.1
CBAE (Chestnut bur autohydrolysis extract)	0.71 ± 0.08	92.33 ± 12.93	1.6 ± 4.5
Negative control (PBS)	0.53 ± 0.15	100	12.1 ± 1.9
Possitive control (SDS)	0.14 ± 0.09	26.28 ± 17.8	503.7 ± 72.1
WBC (Wine byproducts phenolic concentrate)*	0.46 ± 0.09	92.34 ± 17.24	15.6 ± 6.7

*In Díaz *et al*., 2012

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FIGURE CAPTIONS

Figure 1. TOTOX values during accelerated oxidation at 50 °C for (a) avocado cream, (b) massage oil and (c) shower oil, using (O) no antioxidant added (Control), (●) Tocopherol, (◆) WBC, (▲) CBAE, (■) SmEE, (■) SmAE, (◆) TE, (▲) FE. Error bars on chart represent standard deviations.

Figure 2. Turbidity of suncream during accelerated oxidation at 50 °C (O) without antioxidant added, and with (●) Tocopherol, (◆) TE, (◆) WBC, (◇) UlAE, (✳) VE.
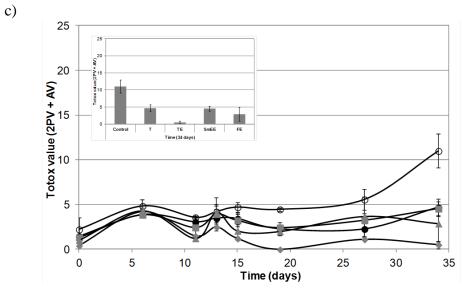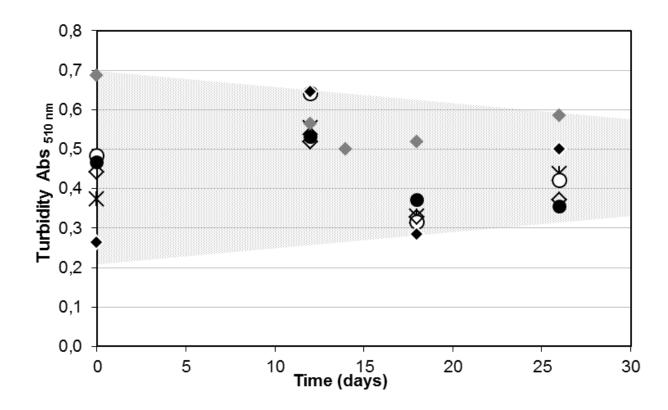
a)

b)

c)

Figure 1

Figure 2