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ORIGINAL RESEARCH

A preliminary study on the use of microalgae biomass as a polyolefin stabilizer

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Abstract

Stabilizers play a crucial role in enhancing the durability of polymers, and recent interest in sustainable materials has accelerated the exploration of natural antioxidants as alternatives to traditional synthetic stabilizers. While antioxidants isolated from natural sources have already been integrated into polymers, the direct introduction of antioxidant-rich biomasses, like microalgae, offers a promising yet less explored approach. This study evaluates the effectiveness of microalgae biomass as a polyethylene (PE) stabilizer, using spirulina (washed and unwashed) and *Tetraselmis striata*. Antioxidant content was assessed through various extraction agents, revealing that water extracts of spirulina contained the highest polyphenol content (940.35 mg/100 g) and antioxidant activity. These biomasses were then integrated into a PE matrix at different loadings (0–30%) and processed by compression molding, while *Tetraselmis* was also processed by rotomolding. Washed spirulina significantly enhanced thermo-oxidative stability in PE, with a 30% loading yielding a 197% increase in oxidation induction time (OIT). *Tetraselmis* also proved to be effective, extending OIT from 0.6 to 44.7 min in compression-molded PE at the highest load. However, *Tetraselmis* did not yield similar results in rotomolding, where no notable OIT improvement was observed. Comparatively, unwashed spirulina provided moderate stability enhancements, though less effective than the washed biomass. This study suggests that microalgae, particularly washed spirulina, are viable eco-friendly stabilizers for PE, opening pathways for more sustainable polymer development.

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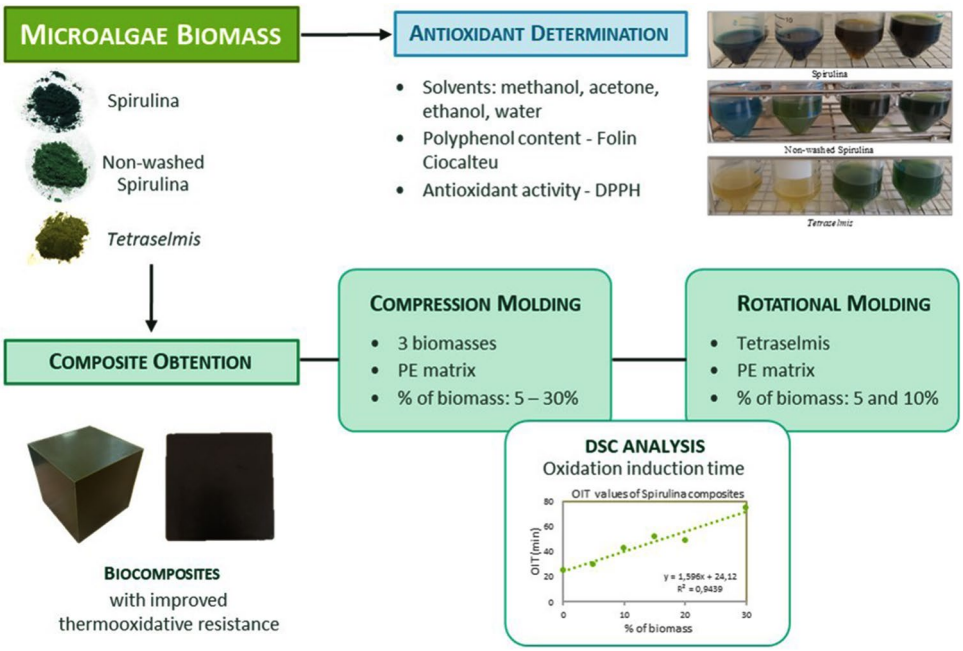
Graphical abstract

A novel amino acid-based hydrogel formed by the self-assembly of Fmoc-L leucine and Fmoc-L lysine , which consisted of twisted nanobelts, displayed antibacterial activity against Gram-positive and Gram-negative bacteria.



Keywords Compression molding · Rotomolding · Natural antioxidant · Polyphenols · Stabilization · Microalgae · Biocomposite

Introduction

Antioxidants are indispensable chemical compounds used in a wide range of industrial applications, as they are able to safeguard the quality and integrity of a wide range of materials, preventing the detrimental effects of oxidation [1]. For example, antioxidants have attracted much attention as food stabilizers, dietary supplements, and natural health products [2], because they play a vital role in both food systems as well as in the animal and human body to reduce oxidative processes and harmful effects of reactive oxygen species (ROS) [2]. But their use is not only limited to biological systems; many other industrial applications that involve materials that are vulnerable to oxidative degradation require antioxidant protection, like for example the fuel or textile industry as well as in applications where paints and coatings are used. The plastic field is another beneficiary of antioxidants, where their use is important as they inhibit polymer degradation during its processing and service life [3]. Polyolefins, such as polyethylene (PE) and polypropylene (PP), the most used polymers, are converted into products at high temperatures [4] and these, added to the exposure to shear forces and the amount of oxygen present during processing, are the main factors determining thermo-mechanical or thermo-oxidative degradation [5]. On the other hand, throughout their lifetime, polymers are exposed to environmental factors (heat, UV light, oxygen, humidity, etc.), leading to the deterioration of their properties; stabilizers are therefore essential additives to enhance polymer durability and stability, providing long-term performances of the material during the service life [6].

Synthetic antioxidants, such as butylated hydroxyanisole (BHA), BHT, and *tert*-butylhydroquinone [7] dominate the market as their production is inexpensive [8]. However, some questions have been raised about the health and environmental hazard of synthetic antioxidants [5] and this, along with sustainability issues (like preference for materials from renewable sources), has led to an increased demand for natural antioxidants, which is expected to continue [9], although the application of natural antioxidants for the protection of polymers is still in its infancy and the information available is limited and often contradictory [5].

Natural antioxidants are produced by natural molecular formations in plants, animals, mushrooms, and microorganisms such as algae and bacteria [10]. Natural antioxidants can be incorporated as pure antioxidant compounds isolated from fruits and plants, with polyphenols being the main class of natural antioxidants used to stabilize polymers

[6]. The review of Kirschweng et al. [5] shows interesting results obtained using natural antioxidants as stabilizers for polymers. Carotene, curcumin, vitamin E, lignin, and other compounds have been shown to have stabilizing effects in different polymers, but mostly in polyethylene [5], while flavonoids seem to be the most efficient natural stabilizers, at least in polyethylene [4], being much more efficient stabilizers than the hindered phenols used in industrial practice [5]. Also, antioxidants can be incorporated as extracts from sources that are rich in antioxidants; some examples are the study of Valero et al., who demonstrated the capacity of grape marc extract and pomegranate peel extract to significantly improve PLA thermal stability during processing and thermo-oxidation [6], or the one by Dopico-García et al., in which green tea extract was able to act as a stabilizer during the performance of polypropylene [11]. Cerruti et al. claimed that the extract of tomato skin and seed improves the processing stability of polypropylene (PP) [12]. Coffee, cocoa, and cinnamon extracts improved polymer stability relating to the weathering aging of PLA, while citrus and green walnut husk extracts enhanced thermal stability [13]. But, compared to these two strategies, a more environment-friendly and cost-effective option for polymer stabilization could be the direct incorporation of an antioxidant-rich material, without a previous extraction step. This last option, chosen in the present study, has received less attention [14]. Examples of this option include for example the polyethylene-based composites obtained by Hejna et al. with spent coffee grounds and cocoa husks, fillers that were able to increase the oxidation induction time (OIT) of the PE matrix [15]. In another study of the same authors, the beneficial effect of the silverskin addition on the thermal properties of the composites was demonstrated, as its incorporation increased the thermo-oxidative resistance of HDPE [16]. On the other hand, black tea waste was able to improve the OIT of rotomolded polyethylene [17], where this work is particularly relevant for the purpose of this study, due to the long cycle times of rotational molding processing and therefore the increased tendency to oxidation and degradation.

In the present study, microalgae biomass has been chosen as a source of antioxidants to be incorporated in a compressed and rotomolded PE matrix. Microalgae have proven to be an interesting source of bioactive compounds, such as essential amino acids, polyunsaturated fatty acids, and antioxidant compounds [18], presenting attractive characteristics such as its fast growth, high biomass productivity, and the ability to synthesize complex metabolites with minimal resources, when compared with higher plants [19]. The publications studying the stabilization potential of microalgae in polymers found in the literature are limited. Mateescu et al. concluded that the use of microalgae (*Chlorella* and spirulina) in PP was able to prevent the degradation of PP, primarily due to the polyphenol content in microalgae [20]. On the other hand, Tafreshi et al. also reported that polyethylene glycol (PEG) combined with *Chlorella* significantly ameliorated the stress caused by gamma irradiation [21]. In a previous work of the authors of the present study [22], the capacity of spirulina biomass to prevent the thermo-oxidation of rotomolded polyethylene was proven.

Although there is evidence to expect that microalgae had a stabilizing effect, the efficiency of the antioxidants depends on different parameters, such as their chemical structure, their concentration (anti-/pro-oxidant effects), the nature of the polymer, or the processing technology, and each matrix – additive combination needs to be investigated [6]. In this study, spirulina biomass, washed and unwashed (as more environmentally friendly and cost-effective alternatives), has been incorporated in the PE matrix using different percentages (0–30%) and processed by compression molding. The influence of its incorporation in the thermo-oxidative stability of the samples, through the measurement of the oxidation induction time, was evaluated. On the other hand, *Tetraselmis* biomass has also been evaluated as a stabilizer of PE; in this case, the composite was processed by compression molding (in percentages of biomass between 0 and 30%) and also by rotomolding (5% and 10% of biomass).

Experimental

Materials

Polyethylene (Revolve N-461 from Matrix Polymers), in powder form, was used as polymer matrix in all the experiments. Polyethylene (PE) has a low melting point, which allows using low temperatures when mixed with the microalgae, thus reducing the risk of biomass thermal degradation.

A. platensis (spirulina) and *Tetraselmis striata* biomasses were kindly supplied by InstitutoTecnológico de Canarias (ITC) in powder format after spry drying. The harvesting of the biomass was performed as described in [23]. In the case of spirulina, the biomass was provided in two different forms: dried washed spirulina (Sp) and dried unwashed material (Sp.N.W).

Determination of bioactive content

Antioxidant extraction

The antioxidants were extracted according to the procedure described by Pérez-Jiménez et al. [24]. In short, 1 g of biomass was extracted for 1 h at room temperature and in the dark with 20 mL of methanol/water (50:50, v/v; pH 2) acidified with 2 N HCl. The sample was then centrifuged at 3000 rpm for 10 min and the supernatant recovered. The residual solid was then extracted with 20 mL of acetone/

water (70:30, v/v) also for 1 h at room temperature and in the dark, centrifuging the mixture after the extraction. The methanol and acetone extracts were combined, the volume adjusted to 50 mL and and filtered for analysis.

On the other hand, mixtures of water and ethanol were also used for antioxidant extraction. In this case, 0.5 g of sample and a solid–liquid ratio of 1:20 (weight of sample/volume of solvent) in 50 mL centrifugal tubes were used for the extractions. The samples were placed in the dark in a thermostatic water bath at room temperature with magnetic stirring; extraction time was fixed in 30 min and ethanol concentrations of 0%, 30%, 60%, and 100% were tested. After the extractions, samples were centrifuged at 3000 rpm for 10 min and the supernatant was filtered for further analyses.

Extractions were performed in two different replicates of each feedstock. Total phenolic compounds (TPC) and antioxidant activity according to the DPPH (2,2-diphenyl-1-picrylhydrazyl) assay were determined in the filtered extracts.

Determination of polyphenolic content

The Folin–Ciocalteu colorimetric method described by Singleton et al. [25] was used to quantify the total phenolic content (TPC) of the extracts. A 0.5 mL volume of blank, standard, or extract was placed into a 25 mL assay and 0.5 mL of Folin–Ciocalteu reagent (VWR) was added. After 5 min in darkness at room temperature, 10 mL of 75 g/L sodium carbonate solution and 14 mL of distilled water were added. The mixture was vortexed and incubated for 1 h at room temperature in darkness. The absorbance was determined at 750 nm using a spectrophotometer (Cary 60 UV–Vis Agilent Technologies). Quantification was done with respect to the standard of gallic acid. The results were expressed as gallic acid equivalents (GAE) in mg per 100 g of dried material.

Evaluation of antioxidant activity by DPPH

In vitro antioxidant activity was calculated using 2,2-diphenyl-1-picrylhydrazy (DPPH) assay according to [26] with minor modifications: 1950 µL of DPPH solution (0.06 mM in methanol) was added to 50 µL of blank or sample extract. The mixture was vortexed and incubated for 30 min at room temperature in darkness. Then, the absorbance was measured at 515 nm and the DPPH activity, expressed in percentage, was calculated according to Eq. 1:

$$\text{DPPH Inhibition}(\%) = (AC - AS)/AC \times 100, \quad (1)$$

where AC is the absorbance of the blank solution containing water and DPPH and AS is the absorbance of the solution containing DPPH with the extract.

Composites preparation

The materials were previously dried at 60 °C for 24 h for PE, and for 6 h for the biomasses. For composites preparation, the matrix (PE) and the biomass were manually blended at the corresponding ratio.

For compression-molded composites, 100 g of the blend was molded in an aluminum mold of 190 × 190 mm using a Collin P200 PM hot press, allowed obtaining 3-mm-thick plates. Previous tests conducted with the microalgae and PE allowed defining the processing cycle: heating to 160 °C at 5 bar for 6 min, followed by a stage of higher pressure (50 bar) for 4 min, and with a last cooling stage, performed under the same pressure.

For rotomolded composites, cubes of 242 g and 3–4 mm of thickness were obtained in an aluminum cube-shaped mold (120 mm side) placed in a laboratory-made rotomolding machine (clamshell type with air convection heating/cooling system). The fan in the oven was set to 1200 L/min and a temperature of 270 °C. The heating stage was ended when the temperature inside the mold reached the desired temperature, fixed in 168 °C after some preliminary tests. The rotation speed was 2.30 rpm in the primary axis and 9.14 in the secondary axis.

The samples have been named with the letter C or R (corresponding to compression or rotational molding), followed by the matrix (PE), the percentage of biomass (5–30% by wt for compression molding and 5–10% for rotomolding), and the biomass (Sp: spirulina, Sp.N.W: non-washed spirulina, TETRA: *Tetraselmis*).

Composites characterization

Thermal characterization

Oxidation induction time (OIT) of composites was determined by the differential scanning calorimetry (DSC) analysis, following ISO 11357–6:2018 standard, in a Q1000 DSC device from TA Instruments. In short, the 10 mg sample was placed in an open aluminum pan and heated from 30 to 210 °C with a heating rate of 20 °C/min in nitrogen, kept at 210 °C for 5 min also under a nitrogen atmosphere, and then, the gas was switched to air/oxygen at 50 mL/min and the time required for sample oxidation was measured. The OIT was calculated as the time to the onset of oxidation subtracted by the initial time under nitrogen.

The differential scanning calorimetry of composites was conducted in a Perkin-Elmer DSC 4000 apparatus (Perkin-Elmer, Waltham, MA, USA). The samples (9–12 mg) were heated from 30 °C to 200 °C under a nitrogen atmosphere at a heating rate of 10 °C/min, cooled down, and heated again to 200 °C under the same conditions. The melting temperature for both heating rounds (T_{m1} and T_{m2}) and

crystallization temperature (T_c) were determined. Cold crystallization was not observed for any of the samples. Melting and crystallization enthalpies (ΔH_{m1}, ΔH_{m2}, and ΔH_c) were used to determine the degree of crystallization using Eq. 2:

$$Xc = \Delta H_{m2} / \left(\Delta H_0 \cdot (1 - mSp)\right) \times 100, \quad (2)$$

where ΔH_0 is the enthalpy for 100% crystalline sample (293 J/g for PE), and mSp is the mass fraction of the microalgae.

Mechanical characterization

The tensile, flexural, and impact properties were determined following well-known standard procedures, namely UNE 527–2:2012 for tensile, UNE 178:2019 for flexural, and ISO 180:2001/A2:2013 for impact testing. Tensile and flexural tests were performed in a LY-1065 universal testing machine from Dongguan Liyi Test Equipment, at a rate of 2 mm/min to determine the elastic modulus, and 10 mm/min for ultimate strength, for both tests. Impact testing was carried out in a LY-XJJD 50 apparatus, also from Dongguan Liyi Test Equipment, using a 5.5 J pendulum and an impact rate of 3.5 m/s. Six specimens were used for each test.

The cross section of fractured specimens was observed in a Hitachi TM3030 SEM table-top microscope, with an acceleration voltage of 15 kV. The specimens were previously sputtered with a thin layer of Pd/Pt in a SC 760 apparatus from Quorum Technologies.

Results and discussion

Antioxidant content

Bioactive compounds include a wide range of chemical compounds whose solubility in different solvents varies, the reason why their extraction is influenced by the type of solvent. In the present study, the polyphenol content was determined in the extracts obtained using different solvents. On the one hand, the combination of methanol and acetone extracts obtained according to the procedure published by Pérez-Jiménez et al. [24] led to obtain polyphenols contents of 353.7 ± 7.7 mg/100 g of Sp, 115.1 ± 9.5 mg/100 g of Sp.N.W, and 148.6 ± 1.2 mg/100 g in the case of *Tetraselmis*, measured through the Folin–Ciocalteu method.

On the other hand, mixtures of water and ethanol were also used for antioxidant extraction. Figure 1 shows the different colors obtained for each extract and Table 1 liststhe total phenolic contents and DPPH activities measured in the extracts. In the case of Sp and Sp.N.W, the extraction in water led to the obtaining of blue extracts; as the percentage of ethanol increased, the blue color of the extracts disappeared and, at the same time, the extracts acquired first a brown and finally a totally green tone for 100% of ethanol extracts. Due to the higher purity of the Sp biomass, which does not contain salts, the intensity of the colors of its extracts was higher than the ones obtained with Sp.N.W. This can also be reflected in the values of TPC and DPPH activity measured that, as can be seen in Table 1, were higher for Sp in all cases. The blue color of the extracts is related to the presence of phycocyanin, which is the main antioxidant of spirulina and that is soluble in water, while ethanol extracts of spirulina had higher carotenoid (orange color) and chlorophyll (green color) concentrations

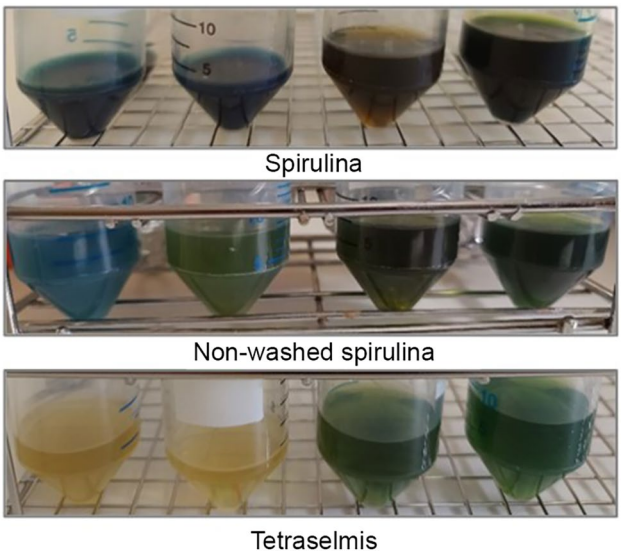


Fig. 1 Extracts obtained under different concentrations of ethanol–water (from left to right: 0%, 30%, 60% and 100% of ethanol) for different biomasses

Table 1 Total phenolic content (TPC) and in vitro antioxidant activity (DPPH inhibition) measured in the different extracts

Microalgae	Solvent	TPC (mg/100 g)	DPPH (%)
Sp	Water	902.38 ± 44.92	33.66 ± 0.64
	30% ethanol	533.59 ± 6.90	Turbid result (not measurable)
	60% ethanol	346.37 ± 39.33	17.27 ± 0.21
	100% ethanol	194.42 ± 14.46	18.76 ± 0.38
Sp.N.W	Water	211.51 ± 5.25	16.36 ± 0.92
	30% ethanol	135.33 ± 2.29	6.76 ± 1.34
	60% ethanol	129.67 ± 7.18	7.39 ± 0.72
	100% ethanol	57.13 ± 2.10	3.24 ± 0.46
Tetra	Water	167.82 ± 9.55	12.57 ± 5.87
	30% ethanol	123.30 ± 3.35	10.74 ± 2.74
	60% ethanol	110.96 ± 0.53	12.73 ± 2.80
	100% ethanol	27.81 ± 0.84	1.20 ± 0.47

compared to aqueous extracts [6]. The highest antioxidant activity was obtained for the aqueous extracts; according to numerous studies, the free radical-scavenging and strong antioxidant activities of spirulina are mostly attributable to the blue-colored phycocyanin [27]. In the case of *Tetraselmis*, the 0% and 30% ethanol extracts presented a pale yellow color, while 60% and 100% extracts an intense green color, although lighter than spirulina extracts. The polyphenol contents of all the extracts were lower than those of the extracts of the other two biomasses.

For the three biomasses, a similar behavior was obtained, the polyphenol content was higher for water extracts and it diminished as ethanol concentration increased. This is in accordance with the results published by Kumar et al. for spirulina extracts, who obtained TPC in aqueous and ethanolic extracts of 9.919 and 3.476 mg/g, respectively [28]; also Goiris et al. [29], working with different species of microalgae obtained the highest phenolic content in the hot water fractions. The high phenolic compounds in the aqueous extract can be correlated with the antioxidant activity. Other authors have also observed a significant correlation between phenolic content and antioxidant activity in microalgae extracts, concluding that when using microalgae biomass as a source of natural antioxidants, not only carotenoids but also phenolic compounds should be considered [28].

On the other hand, it was expected that the method of Pérez-Jiménez et al. [24] allowed to extract more amount of polyphenols due to the use of solvents of different nature (methanol/water and acetone/water), so more compounds could be solubilized. For example, methanol has been generally found to be more efficient in the extraction of lower molecular weight polyphenols, whereas aqueous acetone is good for the extraction of higher molecular weight flavanols [30]. However, for the three biomasses, water resulted in obtaining higher amounts of polyphenols. This can be explained by a degradation of the compounds during the extraction instead of a lack of solubilization power, while the method developed by Pérez-Jiménez can therefore be more suitable for other kinds of biomasses.

Microalgae and cyanobacteria have been recognized as important sources of natural pigments, such as chlorophylls and carotenoids, and, in the case of spirulina, phycobiliproteins for quite a long time [31], and some species of microalgae are already commercially produced as a source of carotenoid antioxidants for use as additives in food and feed applications, as well as for use in cosmetics and as food supplements [6]. Numerous studies have demonstrated that carotenoids contribute significantly to the total antioxidant capacity of microalgae [6] as well as phycocyanin [27]. However, less attention has been given on the presence of phenolic compounds in microalgae and their contribution to the antioxidant activity; where more recently studies have been published in this area. For example, Vieira et al. obtained a TPC of 142.84 mg/100 g on the extracts of spirulina using methanol/water/acetic acid/ascorbic acid (30:67:1:2, v/v/v/w) as extracting solvent and ultrasound assistance; besides, they detected that more than half of the polyphenols were composed of phenolic acids [31]. Da Silva et al., on their part, quantified the polyphenolic content of ethanolic extracts of *A. platensis* via different extraction methods and obtained a maximum content of 3.32 mg/g of dry biomass [32]. Bolanho et al. found that the content of phenolic compounds in spirulina prepared under different conditions ranged from 2.4 to 5 g kg^{-1} [33]. These values are in the same order of magnitude as the ones obtained in the present study. Regarding *Tetraselmis Striata* species, no studies have been found determining the TPC. *Tetraselmis* sp. is widely used in aquaculture as feed because it is a highly adequate source of proteins, carbohydrates, lipids, and fatty acids. *Tetraselmis* species are also very interesting candidates for the production of natural pigments—violaxanthin, lutein, and carotene—for nutraceutical and cosmetics applications [34]. Goiris et al. [29] determined the phenolic content in the ethanol/water extracts of 3.74 mg/g for *Tetraselmis* sp. and 1.71 mg/g for *Tetraselmis suecica*. However, the species *Tetraselmis striata* is one of the lesser studied of the *Tetraselmis* genus [35]; carotenoids and chlorophyll contents have been published in the literature but not polyphenols, so it cannot be compared with the results obtained here.

Characterization of composites: thermal stability, OIT, and mechanical properties

In Fig. 2, the appearance of the original biomasses and the composites obtained by compression molding with the maximum load of the three biomasses can be observed. For the three microalgae, a 30% load allowed to obtain parts with a good appearance and uniform color (different for each biomass as the original material also presented a different color), indicating that a good blending and distribution of the filler was obtained. In the case of *Tetraselmis*, which was also processed by rotational molding, a picture of a cube obtained with 5% of the microalgae is also included in Fig. 2. Although the biomass did not behave as well as it did in compression molding, a good quality surface was obtained for this loading. On the other hand, SEM images of the fracture surface of tensile specimens of 30% of Sp and 30% of Sp.N.W composites are also included; they show a good distribution of the biomass particles along the matrix, with small agglomeration in some areas due to the higher affinity of the biomass particles with themselves than with the matrix. A deeper SEM analysis can be found in a previous work of the authors of this study [35].

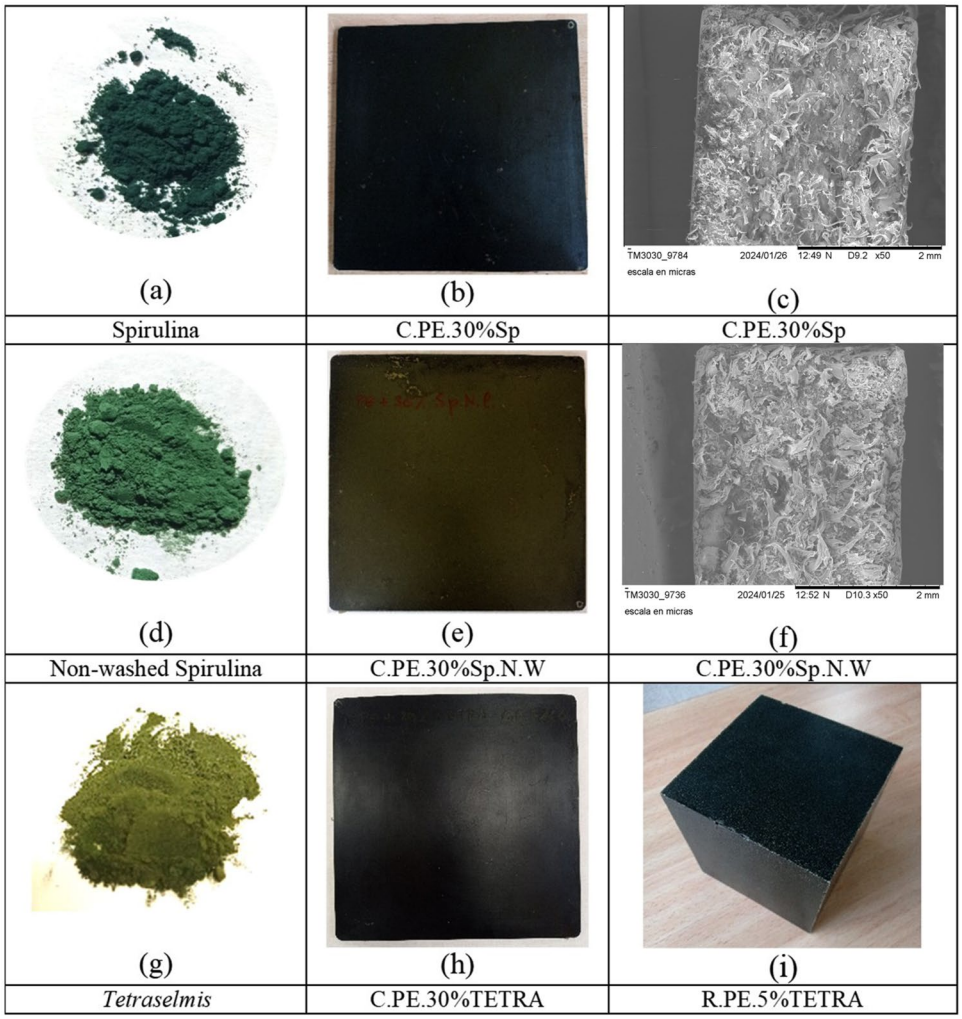


Fig. 2 **a**, **d**, **g** Pictures of the original biomasses, **b**, **e**, **h** composites obtained with 30% of each biomass, **i** by compression molding and 5% of *Tetraselmis* by rotomolding, **c** and SEM micrographs of the fracture surface of tensile specimens of composites obtained with a 30% of Sp and **f** 30% of Sp.N.W

To evaluate the potential of microalgae biomasses as a stabilizer of PE, the oxidation induction time (OIT) of the composite was determined using DSC analysis. OIT is one of the most reliable and commonly used test for the evaluation of polymer thermal stability, especially for polyolefins, and there is a direct correlation between OIT values and stability: higher OIT values involve more stable materials [6]. The OIT results for spirulina-composites are included in Table 2. As can be seen, in general, the OIT values were increased compared with those of PE.

For washed spirulina composites, a correlation exists ($R^2 = 0.94$) between the percentage of biomass and the oxidation induction time, and, for the highest biomass content, an increase of 197% was obtained in the OIT value. In the case of non-washed spirulina composites, oxidation time increased in a similar way (close to 40%) for all composites regardless of the ratio of biomass used; when the highest value was obtained for 20% composites, which allowed obtaining a 56% increase compared to neat PE. The observed behavior is in accordance with the results of the concentration of antioxidants obtained for both biomasses; washed biomass, with higher purity and antioxidants content, allowed to obtain a much higher stabilization effect.

Table 2 Oxidation induction time (OIT) of spirulina and non-washed spirulina compressed composite obtained from DSC analysis

Specimen	Biomass percent (%)	OIT (min)
C.PE	0	25.1
C.PE.5%Sp	5	29.4
C.PE.10%Sp	10	42.8
C.PE.15%Sp	15	51.8
C.PE.20%Sp	20	48.8
C.PE.30%Sp	30	74.5
C.PE.5%Sp.N.W	5	34.2
C.PE.10%Sp.N.W	10	34.6
C.PE.15%Sp.N.W	15	35.0
C.PE.20%Sp.N.W	20	39.2
C.PE.30%Sp.N.W	30	34.2

This is also in accordance with the results obtained in a previous work [22], where Sp and Sp.N.W biomasses were mixed with PE and processed by rotomolding. In that study, the oxidation induction time values were notably increased with the incorporation of the biomass, with this effect being more evident for the washed biomass composites.

The composites here, characterized in terms of their stability, were also characterized in terms of their mechanical behavior. The results of the mechanical properties are summarized in Table 3. It can be concluded that as high as 10% of spirulina (regardless of the use of washed and unwashed biomass) would be introduced in the PE matrix without significant reductions in any mechanical properties compared to neat PE. For this loading, an increase of 71% and 38% was obtained in the OIT value for Sp and Sp.N.W composites, respectively. Considering only the mechanical properties, the previous washing of the biomass could be avoided, as the presence of salts did not affect the material's properties, but in terms of stability it would be positive working with the purified biomass. For higher ratios of biomass, a reduction in mechanical performance was observed, although the molded parts kept good aesthetics and acceptable properties; for example, for the highest stabilization effect, obtained for 30% Sp, no significant differences were observed in tensile and flexural elastic modulus and a slight reduction of the flexural strength (20%) was observed. In the case of maximum tensile strength and impact strength, these properties were more affected and reductions of 62% and 70% were obtained for these properties, respectively. Also in that study, the thermal properties of the composite were evaluated through thermogravimetric analysis and differential scanning calorimetry. Although, as expected, the addition of the biomass resulted in a reduction of the composites' thermal stability, the observed adverse changes did not affect the processability of the material; for instance, the T5% (the temperature at which 5% of weight loss occur) values obtained for the highest loads widely exceed the molding temperature used for the composites' manufacture.

Regarding *Tetraselmis* composites, a different behavior was observed between rotomolded and compressed parts. Table 4 summarizes the values of OIT obtained for both

Table 4 Oxidation induction time (OIT) of *Tetraselmis* compressed and rotomolded composite obtained from DSC analysis

Specimen	Biomass percent (%)	OIT (min)
R.PE	0	0.0
R.PE.5%TETRA	5	1.6
R.PE.10%TETRA	10	1.7
C.PE	0	0.6
C.PE.5%TETRA	5	9.9
C.PE.10%TETRA	10	17.0
C.PE.15%TETRA	15	17.9
C.PE.20%TETRA	20	32.3
C.PE.30%TETRA	30	44.7

Table 3 Mechanical properties of the composites

Specimen	Impact strength (kJ/m^2)	Flexural strength (MPa)	Flexural elastic modulus (MPa)	Tensile strength (MPa)	Tensile elastic modulus (MPa)
C.PE	12.59 ± 0.61	12.38 ± 0.17	488.97 ± 34.26	12.24 ± 0.42	422.05 ± 40.20
C.PE.5%Sp	12.73 ± 0.79	12.32 ± 0.42	551.56 ± 23.58	11.41 ± 0.18	427.15 ± 31.73
C.PE.10%Sp	12.00 ± 0.53	12.37 ± 0.17	559.80 ± 20.00	9.88 ± 0.23	470.35 ± 20.71
C.PE.15%Sp	11.32 ± 0.58	11.69 ± 1.06	509.60 ± 53.25	8.02 ± 0.68	456.30 ± 13.86
C.PE.20%Sp	9.98 ± 2.75	11.18 ± 0.25	511.39 ± 30.70	7.02 ± 0.45	433.07 ± 19.30
C.PE.30%Sp	3.53 ± 1.18	9.91 ± 0.91	649.45 ± 31.04	4.69 ± 0.69	452.96 ± 62.31
C.PE.5%Sp.N.W	12.89 ± 0.27	11.54 ± 0.30	483.83 ± 30.60	11.46 ± 0.28	416.82 ± 23.03
C.PE.10%Sp.N.W	12.61 ± 0.42	11.90 ± 0.244	497.62 ± 67.12	9.98 ± 0.27	416.40 ± 18.40
C.PE.15%Sp.N.W	11.99 ± 0.43	11.93 ± 0.14	507.30 ± 49.13	9.26 ± 0.56	417.00 ± 12.29
C.PE.20%Sp.N.W	9.24 ± 2.89	10.69 ± 1.28	605.03 ± 39.52	6.37 ± 146	400.06 ± 27.92
C.PE.30%Sp.N.W	3.64 ± 1.50	9.61 ± 0.47	539.09 ± 21.15	5.12 ± 0.48	416.06 ± 19.24
C.PE.5%TETRA	12.16 ± 0.40	11.19 ± 0.31	463.46 ± 35.10	10.66 ± 1.33	371.40 ± 49.05
C.PE.10%TETRA	11.65 ± 0.83	11.09 ± 0.51	493.16 ± 46.48	9.83 ± 0.73	396.34 ± 38.69
C.PE.15%TETRA	12.29 ± 0.64	10.57 ± 0.64	523.57 ± 39.30	8.03 ± 0.87	386.00 ± 17.78
C.PE.20%TETRA	10.76 ± 1.13	11.18 ± 0.77	538.06 ± 67.48	6.36 ± 0.71	354.50 ± 37.77
C.PE.30%TETRA	4.38 ± 0.90	9.54 ± 0.43	452.02 ± 65.71	4.36 ± 0.45	281.58 ± 37.33
R.PE	17.19 ± 2.72	16.61 ± 0.94	604.83 ± 52.17	13.89 ± 0.58	400.85 ± 43.39
R.PE.5%TETRA	14.55 ± 1.99	10.71 ± 0.51	403.83 ± 29.29	8.88 ± 0.48	316.58 ± 9.88
R.PE.10%TETRA	8.75 ± 1.09	8.89 ± 0.53	340.57 ± 30.31	6.16 ± 0.39	205.25 ± 20.86

rotational and compression-molded composites containing *Tetraselmis*. In the case of compressed composites, as with spirulina, a significant correlation ($R^2 = 0.97$) was observed between biomass content and OIT, and the oxidation induction time was highly extended for the maximum loading. For rotomolded composites, however, the incorporation of the biomass did not have a beneficial effect on the thermo-oxidative resistance; due to the longer processing time and relatively higher temperature used in this process, the biomass can be more affected. *Tetraselmis*/PE biocomposites can be processed by rotational and compression molding, although this biomass shows poor behavior in rotational molding [36]. A maximum loading of 10% could be used because biomass degradation took place when working with higher ratios, probably due to the high lipid content of *Tetraselmis* [37].

The mechanical characterization of the composites was conducted, obtaining also a different behavior for compressed and rotomolded parts (Table 3). In the case of compression-molded composites, a similar behavior with the obtained one by Sp composites was observed, where as high as 10% of biomass could be introduced in the PE matrix without significant reductions in any mechanical property. For this percentage, the OIT value was considerably extended. If tensile properties are not considered, until a 20% of *Tetraselmis* can be used with the rest of properties practically unaltered. The thermal analysis conducted in the study also showed a reduction in the thermal stability of the composites. In this occasion, the use of maximum 20% biomass did not affect the processability of compression-molded composites, but for higher loadings, the T5% value was significantly reduced. Regarding rotomolded composites, due to the characteristic of the process, they were more sensitive to the introduction of foreign materials, and the mechanical properties were gradually reduced with the percentage of biomass; a 5% of biomass seems to be the limit to obtain acceptable properties. As mentioned before, in most published studies, natural antioxidants have been incorporated as isolated compounds or as natural extracts from plants or herbs that are rich in antioxidants [4]. The direct incorporation of the source of natural antioxidant (as in the present work) has been explored for polymer stabilization to a lesser extent. Although oxidation induction time values are not always directly comparable, due not only to the differences in the processing technology, but also to the conditions in which the oxidation tests are performed (such as the gas atmosphere or flow rate, or the heating rate) affect these values, some results found in the literature are included below for a qualitative comparison purpose. For example, in the work of Nanni et al. [38], three different solid wine by-products (peels, stalks, and seeds) were tested as additives (at 6% by weight) on PP and the OIT was investigated at 175 °C and 200 °C, two temperatures higher than the PP melting temperature, to verify the effect of the additives on thermal stability during the typical processing conditions of PP. The OIT values determined at 200 °C did not change in relation to neat PP, but considerably increased at 175 °C; a maximum increase was obtained for white and red grape peels composites (2.5 min for neat PP versus 22 min for the composite), which reflects the influence of the testing conditions. Other examples are the studies of Hejna et al., who incorporated different by-products in rotomolded PE. The incorporation of antioxidants to prevent the thermo-oxidation of polymers processed by rotational molding is essential due to the long processing times and high temperatures needed in this process, compared to other ones. They found that a 20% of coffee silverskin increased the oxidation induction of HDPE in 120 min [16]. In another study, Hejna et al. observed that the addition of 20% of coffee and cocoa industry by-products extended the oxidation induction time by 100% (54 min) compared to that of pure PE (20 min) [15]. The results obtained in the present study are comparable with those previously published. The considerable stabilization efficiency of microalgae biomass has been proven by oxidation induction time measurement. Although OIT measurement represents a fast and reliable tool to evaluate the antioxidant effectiveness, other tests like long-term thermal stability through infrared spectroscopy (FTIR) and tensile tests could be performed [6]. On the other hand, should a higher effect be desired, the possibility of incorporating an extract of the biomass could be evaluated, which would allow for a higher stabilizing effect with a lower loading. Besides, as shown in the antioxidants content determination section, a simple and environmentally friendly extraction with water could be used, as it allowed in acquiring the highest antioxidant activity in the extract.

Table 5 shows the thermal parameters obtained from DSC analysis of composites; no significant changes of thermal properties (including characteristic temperatures of melting and crystallization) were observed. In relation to crystallinity values, no particular trend in the changes was observed with the percentage of biomass for any of the microalgae.

Conclusion

In this study, the direct use of three different microalgae biomasses, washed and unwashed spirulina, on one hand, and *Tetraselmis,* on the other one, as stabilizer of polyethylene was evaluated using different percentages of biomass (0%-30%) and different processing technologies (rotational or compression molding). First, the antioxidant content determination of the biomasses, carried out using different extractor agents, showed that for all the biomasses, water extracts contained the highest amount of polyphenols and antioxidant activity. Unwashed spirulina was the biomass with higher polyphenols content (940.35 mg/100 g of

Table 5 Thermal parameters obtained from DSC analysis of composites (Tm1—melting temperature 1st heating, Tm2—melting temperature 2nd heating, Tc crystallization temperature, Xc—crystallinity)

Specimen	Tm1 (°C)	Tm2 (°C)	Tc (°C)	Xc (%)
C.PE	128.20	127.71	108.94	43.36
C.PE.5%Sp	127.27	127.06	109.43	49.02
C.PE.10%Sp	127.72	126.91	109.36	30.91
C.PE.15%Sp	128.31	127.04	109.44	43.31
C.PE.20%Sp	127.73	127.01	109.44	38.75
C.PE.30%Sp	127.94	126.61	109.00	40.48
C.PE.5%Sp.N.W	129.14	127.55	108.96	46.09
C.PE.10%Sp.N.W	128.25	126.90	109.61	40.44
C.PE.15%Sp.N.W	128.24	127.36	109.30	39.06
C.PE.20%Sp.N.W	127.98	127.21	109.60	43.81
C.PE.30%Sp.N.W	127.21	126.85	109.78	36.60
C.PE.5%TETRA	128.06	127.08	109.40	36.81
C.PE.10%TETRA	128.19	126.98	109.52	42.80
C.PE.15%TETRA	128.10	126.88	109.42	34.89
C.PE.20%TETRA	128.16	126.93	109.57	43.99
C.PE.30%TETRA	127.30	126.60	109.89	44.01
R.PE	128.56	127.55	108.92	37.01
R.PE.5%TETRA	127.73	126.93	109.57	38.09
R.PE.10%TETRA	128.41	127.10	109.40	39.92

dried biomass) from the ones analyzed in this study. On the other hand, the oxidation induction time determined for the composites demonstrates the potential of washed spirulina biomass as a PE stabilizer. All its composites exhibited substantial improvements in thermo-oxidative stability relative to neat polymer; for the maximum biomass ratio (30%), an increase in the OIT of 197% was obtained. In the case of unwashed spirulina, although a relative improvement in the PE stability was obtained, this biomass did not behave as well as the washed one. On the other hand, *Tetraselmis* biomass also acted as a good stabilizer for compressed PE, allowing the extension of the oxidation induction time of 44 min for a 30% biomass. For rotomolded PE, however, a stabilizing effect was not observed.

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Declarations

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Declaration of generative AI in scientific writing The authors declare that they did not use generative AI systems while preparing and writing the manuscript.

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