



Artubuts unedo L. leaves extract as a potential ingredient for the production of active-films and the preservation of oil-in-water (O/W) emulsions from oxidation

Nisserine El Hammadi^{a,b}, María Pilar Almajano^b, Maria Vicenta Pastor^b,
Idoia Codina-Torrella^{c,*}

^a Laboratory of Materials, Natural Substances and Environment (LAMSE), Chemistry Department, Faculty of Sciences and Techniques of Tangier, P.O. Box 416, Tangier, 90000, Morocco

^b Chemical Engineering Department, Universitat Politècnica de Catalunya, Av. Diagonal 647, 08028, Barcelona, Spain

^c Agri-Food Engineering and Biotechnology Department, Universitat Politècnica de Catalunya, Esteve Terrades 8, 08860, Castelldefels, Spain

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ABSTRACT

Natural antioxidants are of great interest to the food industry to prevent food from oxidation. In line with this trend, this study aimed to investigate the potential application of *Arbutus unedo* L. leaf extracts (AULE) for active-film production and preserving O/W emulsions against oxidation. The best-extracting conditions for the phenolic compounds and flavonoids were obtained with an ethanol:water solution at a 60:40 ratio (AULE60) (~73.28 mg Gallic Acid Equivalents/100 mL and ~27.01 mg Quercetin Equivalents/100 mL, respectively), which was characterized by arbutin, epicatechin, gallic acid and ferulic acid, and exhibited radical scavenging activity. Different alginate-films were also produced. Glycerol decreased their strength and increased their plasticizing behavior, whereas the calcium-crosslinking solution increased their rigidity. The addition of AULE60 resulted in brittle films. The immersion of these films in several food simulants showed that water and acid exhibited the highest values of released polyphenols and radical scavenging activity, in comparison to the ethanolic (20 or 50%) simulants. It was finally demonstrated that O/W emulsions with AULE60 (5 or 10%) or with the AULE60-alginate film exhibited the best oxidative stability over time. Therefore, the potential use of AULE as a natural ingredient for these applications was demonstrated.

1. Introduction

It is known that most food products are perishable and require protection against lipid oxidation and antimicrobial spoilage during their preparation, storage and distribution to extend their shelf life (Vasile & Baican, 2021). Food oxidation, in particular lipid peroxidation, is a complex process that reflects the interaction between molecular oxygen and unsaturated fatty acids (Ayala et al., 2014). This system results in both food spoilage and severe health issues due to free radicals (Lobo et al., 2010). These reactions cause a broad range of oxidation products, such as the hydroperoxides (primary oxidation products) and several secondary products (such as alcohols, ketones, aldehydes, etc.) (Chen et al., 2022). Antioxidants can increase the shelf life of these food products by retarding the lipid oxidation processes. Currently, the high

demand for natural ingredients is driving the food industry to replace synthetic additives with plant extracts, among others, which can act as antioxidants (Dirpan et al., 2023). One of the most important antioxidants in plants is the phenolic compounds, which are characterized as plant-based chemicals that share an aromatic ring with one or more hydroxyl groups (Cosme et al., 2020). These compounds have distinctive properties and functions in plants and other vegetation, such as antioxidant activity, anti-inflammatory and anti-microbial activity and are also used in aroma and food colorants (Alara et al., 2021). Currently, there are about 8000 naturally phenolic structures, which are classified according to their structural complexity and biosynthetic origin (Dai & Mumper, 2010). Flavonoids, some phenolic acids, and condensed tannins share one structure, featuring a C6-C3-C6 ring. Another, simpler C6-C1 structure is found in some phenolic acids and tannins (Zhang, Cai,

* Corresponding author. Department of Agri-Food Engineering and Biotechnology, Universitat Politècnica de Catalunya, Edifici V, c/ Esteve Terrades, 8, CP: 08860, Castelldefels, Spain.

E-mail address: idoia.codina@upc.edu (I. Codina-Torrella).

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et al., 2022). This growing interest makes research focused on integrating these plant extracts directly added into the food-matrix or in packaging materials, as delivery systems, in order to obtain innovative products with antioxidant properties and/or extended shelf-life. This approach aims to achieve two critical goals: creating environmentally friendly packaging and utilizing plant extracts to protect and preserve food products, particularly from oxidation. This dual focus not only promotes sustainable packaging but also leverages the natural power of plants to extend food shelf life and maintain their quality. Among the different ways of incorporating antioxidant agents into active packaging systems, one of the options is the incorporation of these substances into the packaging polymer matrix (Kola & Carvalho, 2023; Tian et al., 2013). In this case, the antioxidants have not been directly included in the food products, but inhibit or retard their oxidation through their release from the film. Recently, biodegradable films tend to be the new evolution of the release system and bio-packaging, as they can offer protection and storage of food products, being aligned with the sustainable trends of the food sector (Baghi et al., 2022). Biopolymers are described to be compatible with several bioactive ingredients. Among them, the alginate is a polysaccharide widely used for the production of these active-packaging solutions (Rangaraj et al., 2021). Furthermore, plant extracts have become a promising solution to include natural bioactive compounds to the packaging materials. In the literature, it has been reported that the type and concentration of these extracts highly determine the characteristics and properties of these bioactive-films (thickness, permeability, mechanical properties, functional activity, etc.), and their biodegradation rate (Kola & Carvalho, 2023; Mir et al., 2018). Among others, extracts of mango peel, peanut skin, banana skin, green tea, etc., have been used to enhance the properties of these films (Rangaraj et al., 2021). Although the antioxidant capacity of these extracts was attributed to several components (such as proteins, carotenoids, saponins, the ascorbic acid, etc.), the polyphenols were described to be the major contributors to the antioxidant activity of these films (Ho et al., 2020; Shan et al., 2023). Additionally, in most cases natural extracts can boost the antioxidant and antimicrobial properties, thus improve the shelf life of packaged food. The use of these extracts as a potent source of antioxidants is an interesting trend, mainly due to that the oxidation of food products are one of the main reasons of their quality deterioration.

Arbutus unedo L. (*A.unedo*), commonly known as the strawberry tree, is a small tree native to the Mediterranean region, which belongs to the Ericaceae family (Bebek Markovinović et al., 2022). Found in southern Europe and northern Africa, this plant is admired for its dark green leaves and edible strawberry-like red berries, which have been studied for their potential health benefits and their content in antioxidant compounds, such as vitamin C and E, the carotenoids, of the polyphenolic compounds (Doudach et al., 2023; Duarte et al., 2023; Zitouni et al., 2022). In the food industry, these plant have attracted particular attention for the production of functional extracts and foods from their fruits and leaves, which could be used in the design of various food products (Bebek Markovinović et al., 2024).

Besides the promising results described in the literature, there is a lot to achieve in relation to the application of some of these extracts to reduce or inhibit one of the main reasons of food deterioration: the oxidative degradation. Thus, this study is a preliminary assessment focused on determining the potential application of *A.unedo* leaves ethanolic extracts (AULE) for the production of active-films and the preservation of O/W emulsions against oxidation. This research aimed to obtain the best AULE, with the highest amount of total phenolic compounds and best antiradical scavenging capacity, with the objective of including this extract into different alginate-films, as an active packaging solution for the food industry to preserve food products against lipid oxidation. The choice of the extracting solvent is decisive as it determines the yield and the selectivity of these bioactive compounds in the final extract. Nowadays, the food industry is focused on reducing the environmental impact of the extraction process, so the use of ethanol

and water as solvents constitutes one of the most ecofriendly alternative (Habachi et al., 2022). In this study, we also aimed to develop different types of alginate-films (with different formulation), to select the best proposal to be included into emulsions. Hence, the mechanical properties of these films and the release behavior of the antioxidants from these polymeric matrices to different food simulants was also evaluated. Finally, the antioxidant activity of AULE was assessed in a more realistic scenario, by assessing the potential of these extracts (and the corresponding active-films) against lipid oxidation of oil-in-water (O/W) emulsions.

This research was aligned with the contemporary trend of seeking natural and sustainable approaches to food production and preservation, responding to the growing popular interest in healthier and environmentally friendly alternatives. Furthermore, this study also promotes the commercial application of plant-derived antioxidants, in particular from a Moroccan variety of *A.unedo*.

2. Material and methods

2.1. Materials

All the analytical grade chemicals, solvents and standards that were employed throughout the entire investigation were acquired from M/S Sigma-Aldrich (Barcelona, Spain) and Thermo Fischer Scientific Co. (Barcelona, Spain). *A.unedo* plant leaves were collected (September) from the northern region of Morocco (35°46'48" N and 5°54'36" W). The leaves were separated and dried (~60 % dry material). Then, the samples were ground (Retsch SM100, Paris, France), sifted through a 1 mm sieve (Gylson Company, Inc, Ohio, U.S.), and stored in plastic containers (at - 80 °C).

2.2. Extraction and determination of total phenolic compounds from *A.unedo* leaves

Plant leaf extracts were acquired through the utilization of ethanol/water at three distinct ratio: 40 EtOH:60 H₂O (AULE40), 60 EtOH:40 H₂O (AULE60) and 80 EtOH:20 H₂O (AULE80) (v/v), according to El Hammadi et al. (2024). Mixtures were stirred in opaque flask glasses (Ovan, MM90E, Barcelona, Spain), for a duration of 6 h. Following this, the supernatant was collected (post-centrifugation for 10 min at 1845×g, and ~24 °C (OrtoAlresa Mod, Consul, Ajlvir, Madrid, Spain)). The resulting extracts were stored (at - 80 °C), in glass vials, until being analyzed. The total phenolic content (TPC) of all extracts was assessed as described by Ouerfelli et al. (2022). The total flavonoids content (TFC) of the extracts was determined following the procedure outlined by Skowrya et al. (2014). Results were expressed as milligrams of Gallic Acid Equivalents per 100 mL of the sample (mg GAE/100 mL of extract), and in milligrams of Quercetin Equivalents per 100 mL of the sample (mg QE/100 mL of extract), respectively.

2.3. Identification, quantification and antiradical scavenging capacity of the phenolic compounds of AULE by HPLC-DAD

The identification and quantification of AULE compounds were conducted through High-Performance Liquid Chromatography (HPLC) analysis, with a Waters Alliance 2695 Series HPLC-DAD equipment (Waters, UK), as described by Bevan et al. (2023). For separating the chemical components, a Water Symmetry C18 column was used (150 mm × 3.9 mm, 5 µm, Waters, UK). Gradient elution was performed using ultrapure water acidified with 0.1% (v/v) formic acid (Phase A) and acetonitrile acidified with 0.1% (v/v) formic acid (Phase B) under the following conditions: 5% B (0–2 min), 5–50% B (2–10 min), 50–95% B (10–15 min), 95% B (15–17 min), 95–5% B (17–18 min), and 5% B (18–20 min). Various commercial standards (arbutin, cinnamic acid, coumaric acid, epicatechin, vanillic acid, ferulic acid, gallic acid, kaempferol, myrcetin, sinapic acid) were employed to identify the

detected compounds, which were in a wavelength set at 280 and 325 nm. The identification of the compounds was confirmed by matching their spectra and retention times (RT) to those of the corresponding authentic standards. Data were analyzed using Empower 2 software (Waters, UK), and the results were expressed in milligrams per liter of infusion.

The radical scavenging activity of the identified individual compounds within the AULE60 was also evaluated through HPLC-DAD, coupled with the ABTS radical post-column injection, as described by Segovia Gómez and Almajano Pablos (2016). The specific details of the HPLC-DAD method corresponded to those described for the identification and quantification of AULE compounds, in the previous paragraph. Positive peaks were detected at 280 nm and 325 nm, using the same DAD detector. The ABTS solution was prepared with 24.24 mM ABTS dissolved in 10 mM solution of PBS. The injection of ABTS^{•+} was executed at a flow rate of 0.4 mL/min through a 3-m-long PEEK tubing with an internal diameter of 0.4 mm. The negative peaks were detected using a UV Detector (2487 UV detector, waters, UK), at a wavelength of 734 nm. Data were analyzed using Empower 2 software (Waters, UK), and the free radical scavenging activity was assessed in comparison to the gallic acid (Le Grandois et al., 2017).

2.4. Films production and characterization

2.4.1. Film preparation

In this study, ten types of films were prepared (with alginate solution, with alginate + glycerol solution, each of which was also produced with and without AULE, and with and without a crosslinking solution (CR) (Fig. 1).

In the case of the films obtained with alginate, they were fabricated as was described by El Hammadi et al. (2024), by dissolving 1.25 g of Alginate 6031 (Brenntag, Spain) and 1.25 g of Vinpai (Cimalgin, France), in 100 mL of distilled water. For the alginate films with glycerol, the film-forming solution was made as described by Farias et al. (2021), which consisted of alginate 6021 (Brenntag, Spain) and Vinpai (Cimalgin, France) at with equal contributions (1.25% w/v) from each. Then, 0.3 g of glycerol was incorporated per gram of alginate. The mixture was stirred in a magnetic stirrer to assure the homogeneity of it. After that, the air bubbles were taken of under vacuum, for 15 min. For

the films with AULE, 25% of AULE60 was added to the film-forming solution (replacing that percentage of the initial water). All these mixtures were sonicated at room temperature (~24 °C) at 15 kHz, in an ultrasonic bath (PROLABO, Merck, Germany), for 20 min. Following the sonication process (Fig. 1), the homogeneous solution was poured into the Petri plates and dried at 40 °C overnight (~16 h). Of each type of these films, a homologous sample was produced with also the CR. The solution was composed of a ratio of 30:70 ethanol:water (v/v), and 3 % (w/v) of CaCl₂, and prepared following the procedure described by Farias et al. (2021). The previously obtained and dried films were immersed for 5 min in 100 mL of this solution and dried for 2 h at 30 °C. Finally, the films were stored at room temperature (~25 °C) in a Petri plate until needed. Of each film, the bottom part was those not exposed to air, but instead in contact with the plate, while the top part was named the *surface* of the film.

2.4.2. Microstructure

The microstructure of the films was analyzed through scanning electron microscopy (SEM) using an FESEM JEOL J-7100 instrument (Oxford Instruments, UK). Small pieces were cut (~2 mm) from the different films: one from the top (surface in contact with the air) and the other from the bottom part (surface in contact with the plate-dish). The samples were directly mounted onto stubs using graphite adhesive. The surface of the films was then scanned to capture microstructural images, following the methodology shown by Amensour et al. (2010).

2.4.3. Mechanical properties

Mechanical properties of the films were determined in a Zwisch Roell Texture machine, mod. Zwicki Z0.5 TN, equipped with a 5 kg load cell (probe S/N 778312)(ZwickRoell, Ulm, Germany). Samples (3 from each film) were cut with a scalpel, in strips of 50 mm length and 10 mm width, and mounted between the grips of the machine. The strain rate was fixed at 5×10^{-4} s⁻¹ and the experiment was done until rupture, recording the stress (σ) and strain (ϵ) curves. Three replicates of each film were tested. The tensile strength, Young's modulus, and Elongation at break values of films were calculated with the TestExpert III software (Zwick Roell, Ulm, Germany).

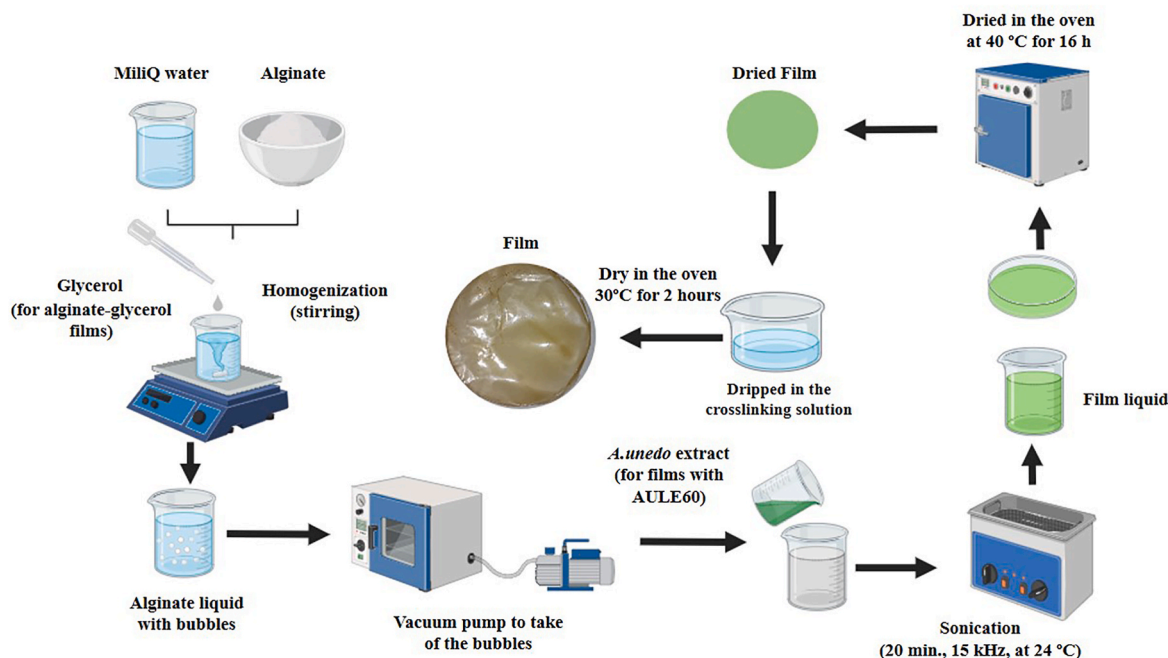


Fig. 1. Flow chart for the production of the different type of films.

2.5. Release of phenolic compounds from films in different food simulants

For the evaluation of the film diffusivity, two types of films with AULE60 (both with and without glycerol) were elaborated, according to Section 2.4.1. The diffusivity of the active compounds was assessed by placing small film pieces (~15 mg) into various vials, each containing 1 mL of different food simulants (20 % ethanol (v/v), 50 % ethanol (v/v), MiliQ water, and 3 % acetic acid in MiliQ water (w/v)). These simulants were selected based on the European Commission Regulation No.10/2011 (EC, 2011), on plastic materials and articles intended to come into contact with food. These vials were then stored at ~20 °C, for 3 days. Samples of the simulants were collected every 10 min within the first hour, each hour for the first 4, 24 and 48 h, and at the end of 3 days. Samples were characterized according to their TPC (Section 2.2.) and radical scavenging activity (Gallego, Skowrya, Gordon, Azman, & Almajano, 2017). Results of Total Polyphenols Content (TPC) released in the simulants were expressed in mg of Gallic Acid Equivalents per L (mg GAE/L) and results of Trolox Equivalent Antioxidant Capacity (TEAC) were expressed as mmol of Trolox Equivalent per L (mmol Trolox Equivalent/L).

2.6. Antioxidant activity of films and extracts in O/W emulsions

2.6.1. Emulsion preparation

The oil-in-water (O/W) emulsion was formulated as follows: Milli-Q water (89 %, w/w), methyl linoleate (MeLo, 10 % w/w) and Tween-20 (1 %, w/w). Emulsions were prepared according to (Bevan et al., 2023). After the dilution of Tween-20 in Milli-Q water using a magnetic stirrer (MM90E, Ovan, Barcelona, Spain) for 1 min (at ~24 °C), the MeLo was added dropwise with continuous sonication using an ultrasonic homogenizer (Hielscher, UP200S, Teltow, Germany) in an ice bath, for 5 min at 30 Hz. The resulting product was distributed into various vials (each with ~13 g). The vials of samples were: Control (sample that only contained the emulsion), 5% AULE60 (sample with the emulsion and 5% of AULE60), 10% AULE60 (sample with the emulsion and 10% of AULE60), alginate-film with AULE60 (sample with the emulsion and AULE60 alginate film) and alginate-glycerol film with AULE60 (sample with the emulsion and AULE60 alginate glycerol film)(see section 2.4.). The films were incorporated into the internal surface of the vial (covering the walls and the base), with a total weight of 0.2 g of film. Each treatment was done in triplicate. For comparison under the same conditions, the total content of ethanol of each vial was adjusted to 6 % (v/v). Thus, the necessary amount of 60 % of ethanol (v/v) was directly added to the control and to the emulsion with films, and in the case of the emulsions with AULE, the percentage of ethanol was adjusted considering the amount of AULE60 contained in the vial. The vials were placed in a dark oven set at 25 °C for 16 days, and different analyses were performed during storage.

2.6.2. Primary oxidation measures (peroxide value, PV)

The primary oxidation reactions in emulsions were evaluated by determining the peroxide value, as described by Gallego et al. (2017). Analysis was performed using a UV-spectrometer (FLUOstar® Omega, Ortenberg, Germany), at 500 nm, over their storage period (14 days). Results were expressed as milli-equivalents of hydroperoxides per kilogram of emulsion (meq hydroperoxides/kg emulsion).

2.6.3. Secondary oxidation reactions (thiobarbituric reactive substances, TBARS)

The assessment of secondary oxidation reactions in emulsions was carried out using the thiobarbituric reactive substances assay, as described by Bevan et al. (2023). Absorbance was measured at 531 nm using a UV-vis spectrophotometer (FLUOstar® Omega, Ortenberg, Germany). Results were expressed as milligrams of malonaldehyde per kilogram of emulsion (mg MDA/kg emulsion).

2.7. Statistical analysis

Data were statistically analyzed using the Minitab® 17 software (Minitab, Inc., State College, PA, USA) by analysis of variance (ANOVA). The Tukey's multiple comparison test was performed to determine the significant differences among samples ($p < 0.05$). The assays and the measures were performed in triplicate. Results were expressed as means $\pm$ standard deviation.

3. Results and discussion

3.1. Obtaining the *A.unedo* antioxidant extracts

In this study, a preliminary assay to optimize the percentage of ethanol used for the extraction of the TPC and TFC from the AULE was performed. As expected, in this work, the recovery of the polyphenolic compounds of *A. unedo* was determined for the ratio water: ethanol of the extracting solvent. *A. unedo* is rich in polar compounds (Jurica et al., 2017), so the variation in the recovery of polyphenol could be highly influenced by the solvent's polarity. Table 1 shows the TPC and TFC of the different extracts of AULE, at the different ethanol:water ratios (40:80, 60:40, and 80:20). The AULE60 and AULE80 exhibited the highest TPC and TFC ($p > 0.05$), whereas the AULE40 was found to have the lowest percentages of these constituents. In all cases, in these extracts, the TFC represented ~35–37% of the TPC. Doudach et al. (2023) reported slightly higher proportion of the TFC of Moroccan *A.unedo* leaf extract (of ~52%), although these authors worked with an extraction solution of 90% methanol, and during 24 h. In fact, aqueous-alcoholic solvents are considered a good choice for the polyphenols extraction (Zhang et al., 2023). Nevertheless, in this case, the increase from 60 to 80% of the ethanol percentage in the extraction solution did not improve ($p > 0.05$) the amount of TPC and TFC. The decrease in the polarity of the solvent could compromise the recovery of the polyphenols from the *A.unedo* leaves, but also the possible strong linkages of these polyphenols with the other components of the food matrix (Zhang et al., 2023). Considering these findings (Table 1), the AULE60 was elected as the optimum extract to continue with the following tasks.

Our findings were similar to those reported in the literature. The main phenol family described in these studies corresponded to flavonoids (Bebek Markovinović et al., 2024), in line with those observed in this study. Other works have described that the TPC of *A.unedo* leaves can range from ~18.7 to ~193 mg GAE/g leaves, although these results depended on the extracting-process and/or the origin of the plant (Jurica et al., 2017; Oliveira et al., 2009; Šic Žlabur et al., 2020). In these works, the recovery of polyphenols was related to the polarity of the extracting solvents. Jurica et al. (2017) reported that the highest amount of total phenols (~96.96 mg GAE/g dry leaf) and tannins (~81.29 mg GAE/g dry leaf) was found in the extract of methanol (at 50%), in comparison to water extract (~66.07 and 54.48 mg GAE/g dry leaf, respectively). Habachi et al. (2022) described that yields of TPC were higher by using 50% ethanolic solutions than those obtained using pure

Table 1

Total phenolic (TPC) and flavonoids content (TFC) of *A.unedo* leaves extract with three different ethanol:water ratios (40:60, 60:40 and 80:20).

Compounds	<i>A.unedo</i> leaves extracts ^b		
	AULE40	AULE60	AULE80
TPC ^a (mg GAE/100 mL)	66.16 $\pm$ 0.10 ^b	73.28 $\pm$ 0.10 ^a	73.53 $\pm$ 0.09 ^a
TFC (mg QE/100 mL)	23.81 $\pm$ 0.07 ^b	27.01 $\pm$ 0.05 ^a	26.93 $\pm$ 0.09 ^a

^{a-b} In the same raw, different superscript indicates significant differences ($p < 0.05$). AULE: *A.unedo* leaves extract obtained with ethanol:water ratios of 40:60 (AULE40), 60:40 (AULE60) and 80:20 (AULE80)..

^a TPC: Total phenolic content; TFC: Total flavonoids content; GAE: Gallic Acid Equivalents; QE: Quercetin Equivalents.

^b Results are presented with the mean $\pm$ standard deviation.

ethanol. Indeed, Orak et al. (2011) reported that the highest TPC of *A. unedo* leaves extracts was found to be in the aqueous extract (5 g of ground leaves mixed with 250 mL of the solvent) (~197.16 mg GAE/g extract), whereas the lowest contents were determined in the pure ethanolic samples (~119.97 mg GAE/g extract). These findings could be attributed to the increase in polarity of the ethanolic solution by the addition of water. Moreover, differences in the phenolic content of *A. unedo* leaf extracts might also be attributed to another factors, such as the methodological conditions of the extraction (temperature, stirring, ratio of ground sample:solvent, etc.), or different extrinsic factors (f.e., the geographical origin, the agroclimatic conditions of the plant, its maturity level, or the harvesting conditions), which could also determine the type of polyphenols contained in the matrix, and therefore, their corresponding affinity for the extracting solvent (Jurica et al., 2017; Malheiro et al., 2012; Oliveira et al., 2009; Orak et al., 2011).

3.2. Phenolic composition and characteristics of the AULE extracts

Table 2 shows the phenolic compounds detected in the AULE using HPLC-DAD. Leaf extracts of this plant have been reported to be characterized by their content in several flavanols (catechins, procyanidin dimers, etc.), flavonols (glucosides of myrcetin, quercetin, kaempferol, etc.), and galloyl and ellagic derivatives, among others (Miguel et al., 2014). Ten different compounds were identified in these plant extracts. In general, as expected, the majority of these compounds exhibited higher concentrations ($p < 0.05$) in the AULE60, while the lowest contents were detected in the AULE40. As shown (Table 2), the arbutin was the main phenolic component of all extracts (AULE60 $\geq$ AULE80 $>$ AULE40), reaching ~119.92 mg/L in the AULE60, ~118.76 mg/L in the AULE80, and ~98.80 mg/L in the AULE40. This glycosylated hydroquinone is widely found in different families of plants, but it was first reported from the leaves of *A. unedo* (Nahar et al., 2022). The arbutin is known for possessing various relevant properties (antioxidant, anti-inflammatory or antimicrobial biological properties) (Habachi et al., 2022; Jurica et al., 2017; Morales, 2022). In all extracts, the epicatechin followed the arbutin in importance, whose concentration was also higher in the AULE60. The remaining phenolic compounds corresponded to coumaric acid, vanillic acid, kaempferol and sinapic acid, which were detected with lower amounts.

In the literature, other authors have described similar results in the leaf extract of *A. unedo* plants (Habachi et al., 2022; Jurica et al., 2017).

Table 2

Amounts (mg/L of infusion) of the different phenolic compounds detected in leaves extract of *A. unedo*.

Compound (mg/L of infusion)	<i>A. unedo</i> leaves extracts ^a		
	AULE40	AULE60	AULE80
Arbutin	98.80 $\pm$ 0.15 ^c	119.92 $\pm$ 0.17 ^a	118.76 $\pm$ 0.09 ^{ab}
Cinamic	19.00 $\pm$ 0.01 ^b	22.07 $\pm$ 0.01 ^a	21.74 $\pm$ 0.26 ^a
Coumaric acid	8.82 $\pm$ 0.04 ^c	11.75 $\pm$ 0.03 ^a	10.68 $\pm$ 0.05 ^b
Epicatechin	50.64 $\pm$ 0.03 ^b	59.19 $\pm$ 0.03 ^a	59.35 $\pm$ 0.14 ^a
Vanillic acid	11.84 $\pm$ 0.01 ^c	15.47 $\pm$ 0.01 ^a	16.01 $\pm$ 0.01 ^a
Ferulic acid	16.63 $\pm$ 0.01 ^b	23.84 $\pm$ 0.01 ^a	23.39 $\pm$ 0.04 ^a
Gallic acid	29.03 $\pm$ 0.01 ^c	36.69 $\pm$ 0.03 ^a	36.32 $\pm$ 0.05 ^a
Kaempferol	8.65 $\pm$ 0.01 ^b	12.90 $\pm$ 0.02 ^a	12.86 $\pm$ 0.02 ^b
Myrcetin	19.91 $\pm$ 0.06 ^b	23.52 $\pm$ 0.04 ^a	24.04 $\pm$ 0.04 ^a
Sinapic acid	9.05 $\pm$ 0.04 ^b	12.09 $\pm$ 0.06 ^a	11.70 $\pm$ 0.04 ^a

^{a-c} In the same raw, different superscript indicates significant differences ($p < 0.05$). AULE: *A. unedo* leaves extract obtained with three different ethanol:water ratios (AULE40, 40:60; AULE60, 60:40; AULE80, 80:20).

^a Results present the mean $\pm$ standard deviation.

Fiorentino et al. (2007) and Jurica et al. (2017) also described the arbutin as one of the most relevant compounds in these extracts, while Fortalezas et al. (2010) reported the gallic acid derivate as one of the most abundant phenolic compounds in the leaves and the fruits of *A. unedo*. Maldini et al. (2019) revealed that in several ethanolic leaf extracts of different *A. unedo* collected in Sardinia, the main flavonoids also corresponded to quercetin, kaempferol and myrcetin derivatives. Intrinsic and extrinsic factors, such as the methodology for the recovery of the compounds, the genotype of these plants, their geographical location and/or the seasonality, among others, might explain the differences among these results (Maleš et al., 2013; Malheiro et al., 2012).

The evaluation of the radical scavenging activity of the individual components was assessed by HPLC-ABTS only in the AULE60, since this extract showed the highest amount of TPC. The determination of the antiradical scavenging activity in this method is based on a decrease in absorbance at 734 nm after the post-column reaction of HPLC-separated phenolic compounds with the ABTS radical cation. Table 3 shows only the phenolic compounds of AULE60 that exhibited a negative peak in the chromatogram.

These results suggested that the most potent free radical scavengers of the AULE60 corresponded to epicatechin (~3.05 mg GAE/L) and arbutin (~2.53 mg GAE/L), with a similar power for stabilizing free radicals ($p > 0.05$). Gallic acid and myrcetin followed them in importance, and showed a similar ($p > 0.05$) radical scavenging potential. As observed in Table 3, coumaric acid exhibited the lowest scavenging activity ($p < 0.05$), probably due to the low amount found, since it has been described as one of the most active free radical scavenging hydroxycinnamic acids (Kiliç & Yeşiloğlu, 2013). As these findings demonstrated, the potential of these bioactive compounds for preventing the formation of free radicals would depend on their chemical structure. The flavonoids have demonstrated to be one of the most powerful agents to capture free radicals (f.e. the flavonols) and chelate metals (f.e. the catechins), whereas the phenolic acids capacity for free radical scavenging would depend on the position and number, in their molecule, of the methoxy and hydroxyl groups (Zhang, Cai, et al., 2022).

3.3. Alginate-based films reinforced with the AULE60 extracts

3.3.1. Film characterization: mechanical properties and SEM-images

Fig. 2 shows the values obtained in the mechanical characterization of all films. The alginate is one of the most used in food packaging and edible coatings (due to its non-toxicity, biodegradability, and its low cost), which is usually mixed with other compounds to modify the film's properties and characteristics (Tavassoli-Kafrani et al., 2016). As observed in Fig. 2, and as expected, films with and without glycerol exhibited different patterns. In general, the alginate-films (without glycerol) exhibited the highest values of the Young's modulus ~2270 – 2495 MPa) and tensile strength (~55.1 – 107 MPa), which indicated

Table 3

Antiradical activity of phenolic compounds of an ethanolic solution (60 %) of *A. unedo* leaves extract evaluated by HPLC-ABTS.

Compounds	Retention time (min.)	Positive peaks ^a	Negative peaks ^a
		Content (mg/L)	Antiradical activity (mg GAE/L)
Coumaric acid	12.8	10.23 $\pm$ 0.08	0.98 $\pm$ 0.01 ^c
Arbutin	2.2	110.80 $\pm$ 0.09	2.53 $\pm$ 0.01 ^a
Myrcetin	15.2	22.97 $\pm$ 0.09	1.21 $\pm$ 0.03 ^b
Epicatechin	11.2	52.48 $\pm$ 0.03	3.05 $\pm$ 0.04 ^a
Gallic acid	4.1	32.14 $\pm$ 0.09	1.31 $\pm$ 0.03 ^b

^{a-b} In the same column, different superscript indicates significant differences ($p < 0.05$).

^a Results present the mean $\pm$ standard deviation; GAE: Gallic Acid Equivalent.

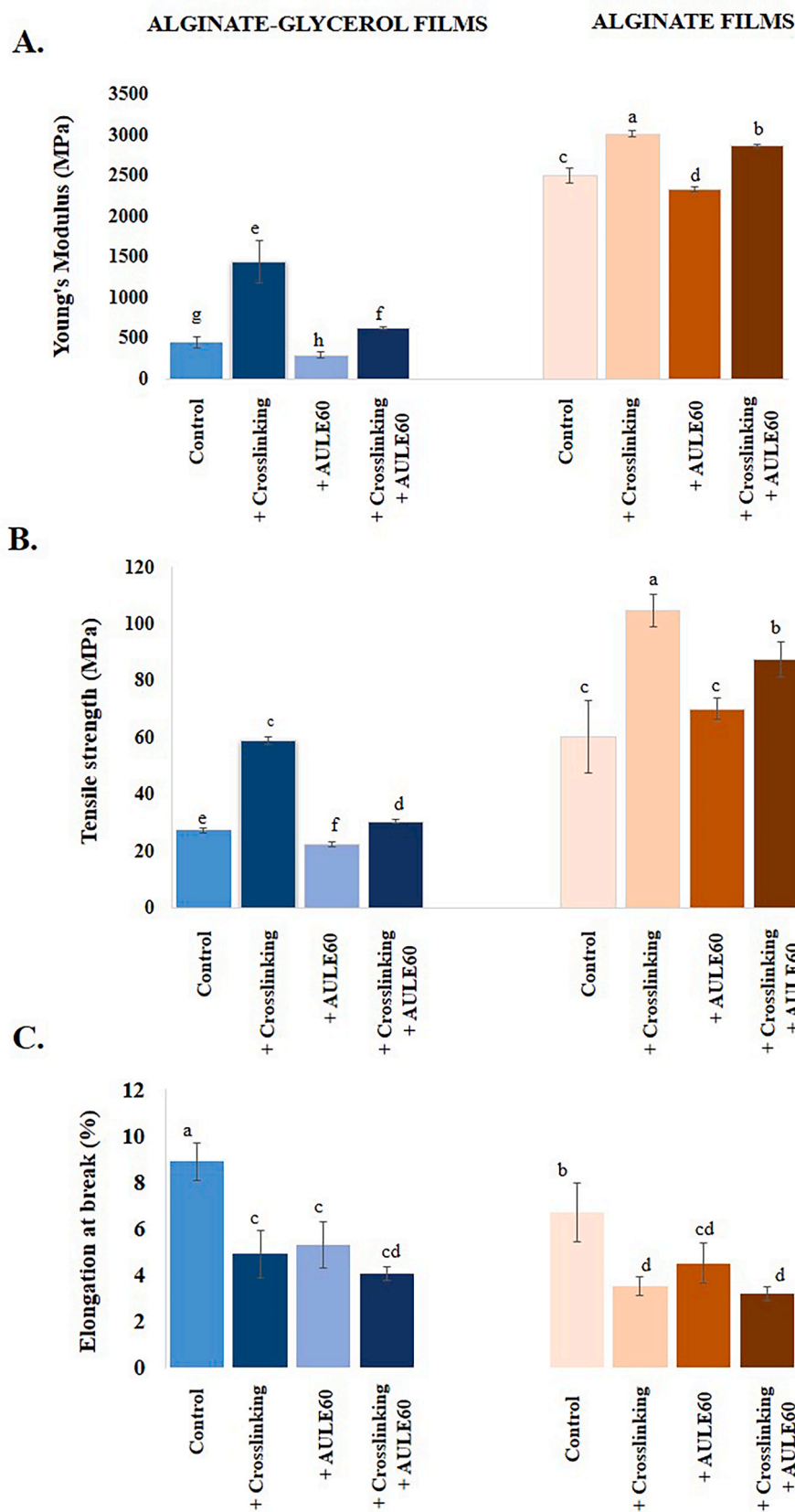


Fig. 2. Mechanical properties of the films. Control: films of alginate (at 2.5%) or films with alginate + glycerol (at 2.5% alginate + glycerol at 30% of the amount of alginate, i.e. 0.75% glycerol); Films with AULE60: films with 25% AULE60; AULE60: *A.unedo* leaves extract with ethanol:water ratio 60:40; Crosslinking solution: 30% (v/v) of ethanol and 3% (w/v) CaCl_2 .

their higher resistance to elastic deformation under an external force. In the film production, the alginate is known for its filmogenic properties, since this hydrocolloid can form fibrous structures in its solid state (Blanco-Pascual et al., 2014). All formulations with glycerol resulted in a decrease ($p < 0.05$) in the Young's modulus ($\sim 550 - 1507$ MPa) and the corresponding maximum tensile strength values ($\sim 22.37 - 59.70$ MPa) of all films (Fig. 2), which means that the maximum force that the films can withstand before failure was lower. Probably, the glycerol contributed to the plastizing effect of the matrix (Gao et al., 2019). Our findings also demonstrated that, in general, the matrix with glycerol showed higher values of the elongation at the break, in comparison with their homologous without glycerol (Fig. 2). These findings might be related to a segregation phenomenon in these matrices due to the addition of glycerol, thus increasing the mobility of polymer chains and the flexibility of

the film (Su et al., 2010).

On the other hand, the addition of the CR in both types of films (alginate and alginate + glycerol) increased their maximum tension force and Young's modulus ($p < 0.05$) values, being these films more rigid. At any case, films with glycerol showed lowest values of both parameters ($p < 0.05$). The divalent metal cations (Ca^{2+}) contained in the CR probably enhanced the chelation of the carboxylate and hydroxyl groups of the alginate, and hence, a more densely cross-linked structure could be formed. These films also exhibited a lower elongation values, in comparison to their homologous without the CR (Fig. 2).

The addition of the AULE made that the force required to cause the film's failure decreased, at any case (films with and without alginate). As expected, AULE-films with glycerol exhibited lower values in Young's Modulus and Tensile strength, in comparison to the film without

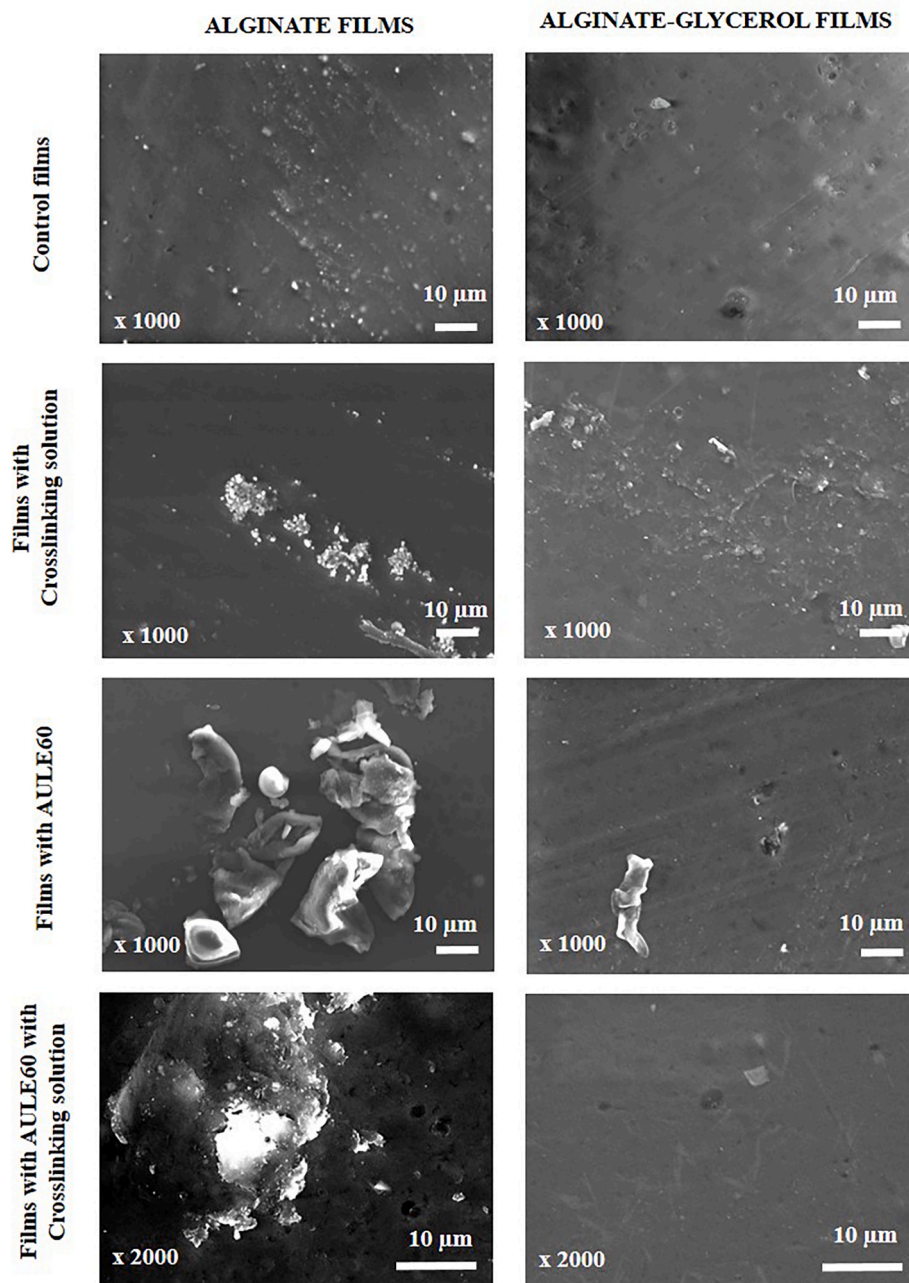


Fig. 3. Scanning electron micrographs (SEM) of top surfaces images of films. Control: films of alginate (at 2.5%) or films with alginate + glycerol (at 2.5% alginate + glycerol at 30% of the amount of alginate, i.e. 0.75% glycerol); Films with AULE60: films with 25% AULE60; AULE60: *A.unedo* leaves extract with ethanol:water ratio 60:40; Crosslinking solution: 30% (v/v) of ethanol and 3% (w/v) CaCl_2 .

glycerol ($p < 0.05$) (Fig. 2). In these films, differences among the films with and without the CR decreased, which suggested that the inclusion of infusions would neutralize the effect of the chelation in both matrices, and also have homogenized the mechanical properties of the films. In fact, it has been previously described that the extracts with antioxidants could influence the mechanical properties of films (Leyva-Jiménez et al., 2024; Yu et al., 2015). Since the hydroxyl groups contained in the polyphenolic compounds could establish intermolecular hydrogen bonds with the components of the matrix (i.e. the proteins, polysaccharides or the plasticizers), by resulting in a more dense and compact matrix. But also more brittle, probably due to the reduction of the intermolecular interactions within the polymeric network of the film (Zhan, Guo, et al., 2015). Nevertheless, Leyva-Jiménez et al. (2024) reported that the incorporation of lower percentages of gallic acid ($<0.5\%$) did not change the Young's modulus value of alginate + methylcellulose + glycerol films, whereas at higher percentages, films resulted less elastic. Other authors also described films with antioxidants with higher values of tensile strength and modulus of Young (López De Dicastillo et al., 2016; Yu et al., 2015). In our case, the interaction between the bioactive compounds of the AULE might enhanced the hydrogen networks with the polymeric chains of the CR, and probably reduced the chelation interactions with the alginate and the water. Samples with AULE lost their deformability and became more brittle, probably due to the decrease in the cohesion forces inside the matrix and the disruption of intermolecular interactions within this network (Zhan, Guo, et al., 2015). Thus, in general, the glycerol decreased the strength of the alginate-films and increased their plasticizing behavior, the CR decreased the flexibility of the polymer chains, and the addition of AULE60 reinforced their brittles.

These findings were consistent with the observations made during the SEM analysis, in which it was observed that the different composition of films affected the appearance of their surfaces (Fig. 3). In general, the bottom layers (part in contact with the Petri dish, see Section 2.4.2.), resulted in a more homogeneous and smoother surfaces than the top-layers (part in contact with the air) (*Images of the bottom surfaces are not presented*). As observed, the film elaborated with only alginate (Fig. 3) showed a less homogenous top surface (with some pores), while on the contrary, the alginate-glycerol exhibited a more homogeneous layer. These differences could be attributed to the presence of glycerol, which might affect the crystallization of alginate granules, increasing the mobility of their chains, promoting their crystallization (Cao et al., 2009). The structural reorganization of the alginate chains could be enhanced with the glycerol, which might limit the formation of visible particles, as well. On the contrary, the alginate-films with glycerol resulted in the presence of smaller and less particles in their structure (Fig. 3), although these irregular shapes were also appreciated on the top-surface.

As observed in Fig. 3, with the CR, all films resulted with changes in appearance. The films of alginate resulted in the constriction of their borders and also presented higher number of larger particles (with irregular shapes) on their surface, likely attributed to traces of the incompletely integrated infusion (Fig. 3). These junctions could be also justified by the direct mixing of alginate and the divalent metal cation (Ca^{2+}) of the CR. The rapid interaction of both components could produce an irreversible formation of larger particles of these two components, resulting in a non-homogeneous gel. In films with glycerol, the addition of this compound probably facilitated the dissolution and the homogeneous integration of the CR with the other components of the matrix, promoting the formation of films with smoother and homogeneous surface.

In all cases, after incorporating the AULE60, it was noticed the existence of some elements of the plants which were not fully integrated into the mixture, resulting in distinct chunks on the film's surfaces (Fig. 3). Nevertheless, and instead of these chunks, these images exhibited the lowest number of porous and irregularities, probably due to the covalent bond interactions of phenolic compounds and the

polymeric matrix that enhanced the homogeneity of film's surface. In fact, Leyva-Jiménez et al. (2024) also described that the addition of gallic acid in hydrogels of alginate + methylcellulose + glycerol resulted in a more compact network with an uniform and smooth internal structure, with less free pore spaces. As far as it is concerned, the characteristics of the films could compromise the diffusivity of the bioactive compounds, so the films with a smoother surface and with less pores tend to exhibit lower and slower release of the bioactive compounds from the film (Dammak et al., 2021). However, other external factors could determine the release of the bioactive compounds, such as f.e., the physicochemical characteristics of the packaged-food product.

3.3.2. Release of bioactive compounds of AULE60 films in different types of simulants

In this assay, all the films that we used corresponded to the films (alginate and alginate + glycerol) with the CR. Films without the CR were discarded to continue with the study, since they lost their structure when immersed in the food simulants. The release of the bioactive agents to ensure the quality and preservation of the packaged food is an indispensable characteristic of an active film (Rangaraj et al., 2021). In our case, these release study was previously performed to ensure the possible potential of these bioactive-polymers from preventing food from oxidation. As reported, several factors determine the release of these bioactive compounds from the films, among which are the characteristics of the extract (particle size, polarity of the bioactive compounds, etc.), the biopolymeric characteristics of the matrix, the interaction between these matrix and the bioactive compounds, etc. (Reis et al., 2023). In this study, the migration of the antioxidant compounds from the film was evaluated concerning the type of film-matrix and also the polarity of the external medium. Fig. 4 shows the release of polyphenols from the films containing the AULE60 across the different food simulants, and the corresponding radical scavenging activity of these simulants over storage. According to the European Commission Regulation No.10/2011 (EC, 2011), the acetic acid 3% (w/v) and ethanol 20 and 50% (v/v) simulants were assigned for foods that have a hydrophilic character and were able to extract hydrophilic substances, specifically, for foods that have a pH < 4.5 (acetic acid simulant), alcoholic foods with an alcohol content $< 20\%$ and/or those with ingredients that render the product a lipophilic character (20% ethanol), or alcoholic foods with $> 20\%$ alcohol or O/W emulsions (50% ethanol). The simulant with only MilliQ-water was used to simulate aqueous-based foods. As expected, based on the type of simulant, different patterns in the release of the polyphenols from the film to the simulant were observed. These findings might be explained to the solvent's polarity and the subsequent affinity of the phenolic compounds contained in the AULE60 to the food simulant. As shown in Fig. 4-A and 4-B, for both types of films (alginate and alginate + glycerol, respectively) the first phase (occurred during the first hour) was characterized for the highest velocity, probably due to the release of the compounds located closer to the surface of the film. This phase was followed by a phase in which occurred a deceleration of this velocity, which could correspond to the release of the molecules inside the polymeric matrix, which presented a slow diffusion due to their interactions with the polymers of the film (Boostani & Jafari, 2021).

As observed in Fig. 4, films immersed in the acetic acid and water exhibited a similar behavior ($p > 0.05$), which was characterized by the highest velocity of release and the highest amount of released polyphenols over the storage of these samples. Although the kinetics of release in water and acetic simulants were not statistically different ($p < 0.05$), there was observed a trend of higher releases in the acetic solution, in both types of films. At 72 h of storage, the TPC in these simulants (acetic or water) corresponded to ~ 191.5 and 203.5 mg GAE/L for the alginate film, respectively, and ~ 199.12 and 190.14 mg GAE/L for the alginate-glycerol film, respectively. Results demonstrated that the type of film had a lower impact on the release of polyphenols in water of acetic simulants during this period of time. These findings were in line

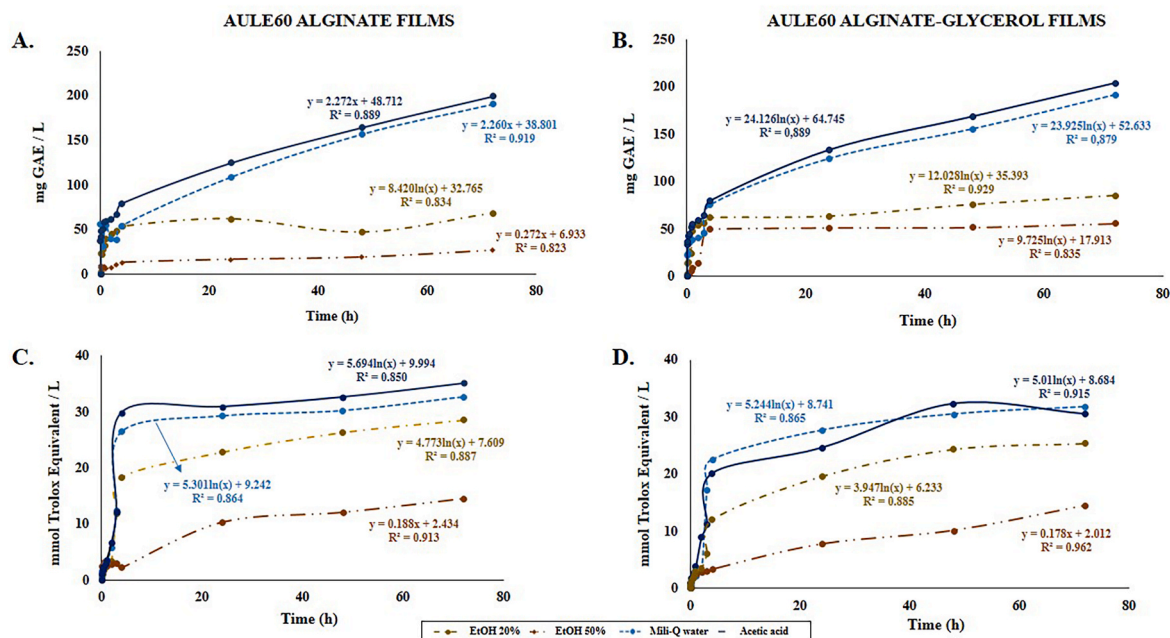


Fig. 4. Release of polyphenols and radical scavenging activity (measured as antiradical scavenger activity against the ABTS) of the AULE60-alginate films (A, C) and AULE60-alginate-glycerol films (B, D) in different food simulants. GAE: Gallic Acid Equivalents. AULE60 alginate films or AULE 60 alginate-glycerol films: films of alginate (at 2.5%) or films with alginate + glycerol (at 2.5% alginate + glycerol at 30% of the amount of alginate, i.e. 0.75% glycerol, respectively, with 25% AULE60 and crosslinking solution (30% (v/v) of ethanol and 3% (w/v) CaCl_2); AULE60: *A. unedo* leaves extract with ethanol:water ratio 60:40.

with those observed in our previous studies (El Hammadi et al., 2024). In this research, *Myrtus Communis* extract was incorporated into an alginate film, which was subsequently immersed in the same food simulants of the current study. We also observed that the acetic acid and water simulants showed the highest content of polyphenols.

In contrast, the release of polyphenols from the films in simulants of 20 and 50% ethanol exhibited different patterns, which were characterized by a slower and gradual increase in the kinetics of polyphenols' release over time. In this case, the release of bioactive compounds of both types of films (alginate and alginate-glycerol) was similar in the EtOH20% simulant ($p > 0.05$), whereas in the EtOH50%, the release was to some extent faster and higher in the alginate-glycerol film ($p < 0.05$).

In general, these findings might be explained by the differences in the polarity of these simulants. In the literature, other authors also stated that the release rate of bioactive compounds encapsulated in films also decreased according to the ethanol concentration in the simulant (Zhang, He, et al., 2022), which also attributed to the solvent polarity decrease. Our findings demonstrated that with independence of the type of film, in general, films that were in contact with water and acid environments exhibited a higher release of polyphenols ($p < 0.05$) than those that were in contact with the ethanolic simulants. The acetic acid, with their capacity of protonation of functional groups of polyphenols could better dissolve these compounds from the film (Coelho et al., 2022), which might be positive to guarantee the satisfactory release of polyphenols in packaged products with pH below 4.5 (f.e. juices). These findings might also suggest that, instead of for watery food-products, for packaged hydrophilic products the release of polyphenols from the film might be granted, although in a different extent according to the intrinsic characteristics of the these products (pH, hydrophilic character, etc.).

The release of polyphenols in these four simulants (Fig. 4) was aligned with the radical scavenging activity of the samples. As observed in Fig. 4-C and 4-D, over the first 50 h, the highest antiradical activity was observed in the acetic and the water simulants for both types of films ($p > 0.05$). On the contrary, simulants at 20% and 50% of ethanol containing films of alginate or alginate-glycerol exhibited a lower antiradical activity, which was to some extent higher in the EtOH20%

simulant, in comparison to the EtOH50% sample. In general, no differences were observed in the antiradical activity of these simulants regarding the type of film (alginate or alginate-glycerol), although the alginate films showed a tendency of higher releases in both ethanolic mediums (Fig. 4C). These findings indicated that with independence of the type of film, the EtOH50% simulant exhibited the lowest antiradical activity ($p < 0.05$), which suggested that these films might exhibit the worst antioxidant potential in alcoholic foods with an alcohol content above 20 % or in O/W emulsions (EC, 2011). At this concentrations of ethanol, the greater resistance to migration of the antiradical substances might be advantageous if a slower and longer release was desired over time.

As observed, the scavenging capacity of these films exhibited their remarkable potential as hydrogen donors to react with free radicals (and converting them into more stable compounds). Considering the radical scavenging activity of these compounds in the different food simulants, in general, as polyphenols dissolved in the medium increased, a higher antiradical scavenging activity was observed in the medium. Even so, the alginate-film immersed in the 20% ethanol simulant exhibited a similar antiradical behavior than the observed in water and acetic simulants ($p < 0.05$), even if containing lower amounts of released polyphenols. The type and amount of polyphenols released in each one of these mediums could explained the current results, since depending on the structure of the polyphenol, they exert a different tendency to scavenge free radicals. In fact, as observed in previous sections, the radical scavenging activity of the AULE60 was mainly represented by different types of polyphenols. Epicatechin (a flavonol) exhibited the highest scavenging activity, and had been reported to be easily solubilized in polar environments (Jurica et al., 2017), which might explain the higher antiradical potential of the 20% ethanol simulant, rather than the amount of released-polyphenols were not the highest. Arbutin (a hydroquinone derivative) was described to be higher in aqueous extracts rather than in methanolic (Jurica et al., 2017). On the contrary, the myrcetin (a flavonoid) or the gallic acid (an hydrolysable tannin) that were also found, in a minor extent, to be responsible of the radical scavenging activity of the AULE60, might be more easily solubilized in acid or water simulants (Zhang et al., 2018), and might have a lower

antiradical potential than the epicatechin or the arbutin (see Table 3). These findings suggest that the radical scavenging potential of the AULE60-films might be highly determined for the characteristics of the packaged-foods, since it would determine the amount and the profile of the released polyphenols, and consequently, the antioxidant potential of this bioactive-film. Other authors have also reported that the antioxidant activity of the films (which contained bioactive compounds) in the emulsions that contained bioactive films increased. In the literature it has also been demonstrated that the antioxidant activity of these films was highly determined by the characteristics and composition of these film, since the release of the active compounds to a higher or lower extent might depend on their linkages with the components of the polymeric matrix (Roy & Rhim, 2021; Sun et al., 2020; Zhang et al., 2022).

Considering these results, it was suggested that if using these active-films as packaging for preventing food against oxidation and extending their shelf life, it should be taken into consideration the composition of the film but also the characteristics of the packaged-food.

3.4. Oxidative stability of oil-in-water emulsions with AULE60-films

3.4.1. pH value of emulsions

The oxidative stability of the oil-in-water emulsions with AULE was assessed in different types of samples. Their initial pH corresponded to 3.12 ± 0.02 for the Control, 3.7 ± 0.18 and 3.52 ± 0.14 for the samples with films with AULE (alginate and alginate-glycerol films, respectively), and 3.27 ± 0.02 and 3.46 ± 0.02 for the samples with AULE (at 5 and 10 %). These pH values gradually decreased over the course of the experiment, without showing remarkable changes ($p > 0.05$).

3.4.2. Primary and secondary oxidation products

In order to evaluate the primary oxidation of the emulsions, the peroxide value (PV) of all samples was measured throughout 11 days (Fig. 5). As observed, all samples showed a constant rise in the PV from the beginning to ~6 days of storage, which corresponds to the induction period. From that moment, a significant difference in the oxidation rate was observed in the Control and in the emulsions with the alginate-glycerol films with AULE and CR. As expected, the Control was faster oxidized over time, which was attributed to the lack of antioxidants as preservers of lipid oxidation reactions.

Unlike what was expected, the emulsion with the alginate-glycerol film with AULE60 exhibited a similar pattern to the Control, implying that the transfer of bioactive compounds from the film to the emulsion has not been able to stop the formation of compounds from primary oxidation, such as the hydroperoxides. This might be due to several factors, but the hypothesis was that, in this system, the polyphenols were retained more in the film due to the presence of glycerol, without the capacity to be released. The final values reached in both cases were ~50 meq hydroperoxides/kg emulsion and were not significantly different ($p > 0.05$). On the contrary, the O/W emulsions with the alginate films showed a different behavior, similar to that of the emulsions with 5 and 10% of AULE60 ($p > 0.05$). These results differed slightly from those obtained in the release of polyphenols from both types of films in the 50% Ethanol simulant (which would correspond to the O/W emulsions (EC, 2011)). In the experiment with this simulant (See section 3.3.2.), the glycerol-film showed slightly higher, but not significant antiradical activity, in comparison to the simulant with the alginate-film, without glycerol. In consequence, these results might suggest that it is more accurate to perform these tests and validations in real-systems. At the end of the storage (~11 days), the emulsions with 5 and 10% AULE60 and with alginate film showed the lowest PV (approximately 20 meq hydroperoxides/kg emulsion). None of these three emulsions were significantly different ($p > 0.05$). This could be explained by the existence of sufficient amount of antioxidant compounds in these three emulsions (either directly added or obtained by diffusion), which could be attributed to the antioxidant activity of the bioactive compounds

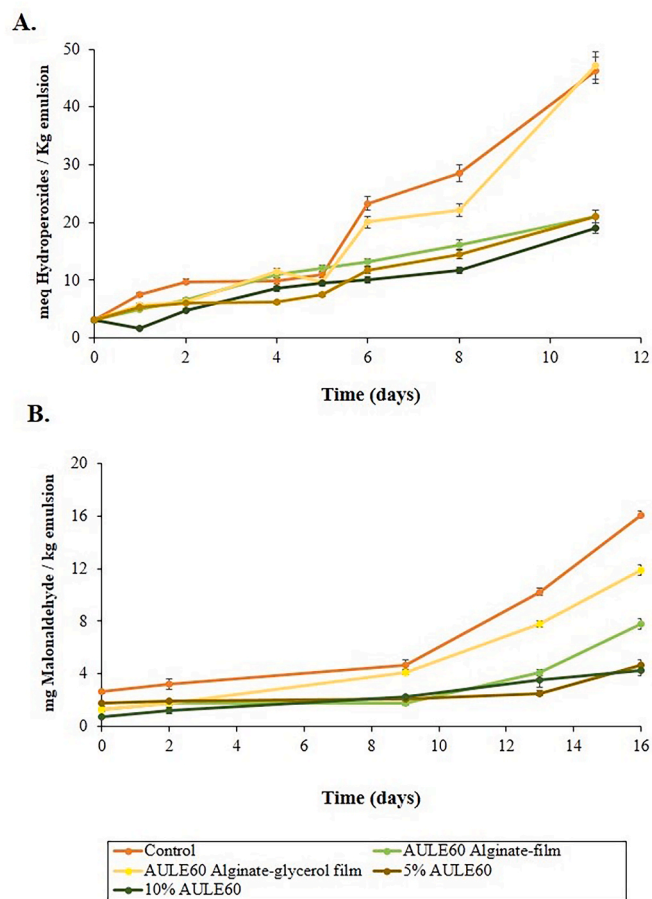


Fig. 5. Evaluation of (A) peroxide value and (B) Thiobarbituric acid reactive substances (TBARS) of oil-in-water (O/W) emulsions during their storage. Control; emulsion; AULE60 alginate-film: emulsion with AULE60-alginate film (at 2.5% of alginate with 25% AULE60 and crosslinking solution); AULE60-alginate-glycerol film: emulsion with AULE60 alginate-glycerol film (at 2.5% alginate + glycerol at 30% of the amount of alginate, i.e. 0.75% glycerol, with 25% AULE60 and the crosslinking solution); 5 and 10% AULE60: emulsions with 5 or 10% of AULE60; AULE60: *A.unedo* leaves extract with ethanol:water ratio 60:40; Crosslinking solution: solution of 30% (v/v) of ethanol and 3% (w/v) CaCl_2 .

contained in the extract, and their corresponding antiradical scavenging activity (Table 3). These results suggested that with a lower percentage of AULE60, the protection of these emulsions against lipid oxidation could be equally effective. At this time, the emulsion with the alginate-AULE60 film showed a PV of ~18.65 meq hydroperoxides/kg emulsion. Considering these results, we expected that the polyphenols contained in the AULE60 films could effectively be diffused into the O/W emulsions, and undergo one-electron and hydrogen transfer mechanisms to protect the O/W emulsions from lipid oxidation during their storage.

Fig. 5 presents the secondary reactions observed during 16 days of storage. Initially, all the emulsions exhibited similar values of TBARS, and only the Control (with ~2.60 mg MDA/Kg emulsion) differed significantly from the others (~0.81–1.73 mg MDA/kg emulsion). With time of storage, and as expected, the Control emulsion showed the highest increase of products resulting from the secondary reactions of oxidation (with 16.10 mg MDA/kg after 16 days of storage). In this case, the emulsion with the alginate-glycerol film followed the control in importance (with ~11.89 mg MDA/kg emulsion) ($p < 0.05$)(Fig. 5-B). As observed in the primary reactions of oxidation (Fig. 5-A), the phenolic compounds released from the alginate-glycerol film to the emulsions were not sufficient to maintain the oxidative stability of the

emulsion, and did not allow delaying the formation of secondary oxidation compounds. In contrast, the other emulsions (emulsions with alginate-films, and the AULE60 extracts) remained stable over time, with similar ($p > 0.05$) values of TBARS (≤ 5 mg MDA/kg emulsion), $\sim 30\%$ less than the others. No significant differences ($p > 0.05$) observed among these emulsions demonstrated again the potential of the alginate-film with AULE60 for the preservation of O/W emulsions against oxidation. Regarding the O/W emulsions, other authors also reported the improvement of the oxidative stability of these systems after the incorporation of plant extracts (f.e. *Rosmarinus officinalis*, *Mentha piperita*, *Thymus vulgaris*, *Caesalpinia decapetala*, etc.), due to their content in polyphenols (Kim & Choe, 2016; Malinowska et al., 2014). These studies also demonstrated that the antioxidant potential of these extracts improved in accordance to the concentration of the plant extract incorporated to the emulsion, and also confirmed that the phenolic compounds in the plants extracts have the ability to preserve O/W emulsions from oxidation. Overall, the bioactive films with plant extracts rich in antioxidants might represent a promising approach to enhance the O/W emulsion's stability, by improving the overall quality of the product without the necessity of the addition of these bioactive-extracts directly mixed to the matrix.

4. Conclusions

The study demonstrated the potential application of *A.unedo* leaf extracts as natural ingredient for the preservation of O/W emulsions against lipid oxidation, and the production of active-films for food packaging. The extraction at a 60:40 ratio of ethanol:water yielded the best extraction of the total phenolic (~ 73.28 mg GAE/100 mL) and flavonoid (~ 27.01 mg QE/100 mL) compounds. All extracts were mainly characterized by their content in arbutin, epicatechin, myrcetin and gallic acid, which exhibited antiradical activity. Regarding the film's composition, the glycerol decreased the strength of the alginate-films and increased their plasticizing behavior and homogeneity, whereas adding the calcium-crosslinking solution increased their rigidity. The addition of the AULE60 resulted in films with less porous, more brittle and with irregularities in their surface. Furthermore, our findings indicated that the release of polyphenols from the films of AULE60 mainly depended on the characteristics of the simulant in which the film was immersed, rather than film's composition. The immersion of these films in the food simulants showed that, after 72 h, the highest release of polyphenols and radical scavenging activity was observed in water and acetic simulants, whereas in the ethanolic simulants (20 or 50%), the release of polyphenols was inversely proportional to the percentage of ethanol. Finally, it was demonstrated that O/W emulsions with the addition of AULE60 improved their oxidative stability over time. At the end of the storage (~ 16 days), the emulsions that contained the AULE60 extract (5 or 10%) or the alginate-films with AULE60 exhibited a better oxidative stability (with ~ 21 meq hydroperoxides/kg emulsion for PV and ~ 4.60 mg MDA/kg emulsion for TBARS), followed by those with the AULE60-alginate-glycerol films. Furthermore, this findings reinforced the existing data about *A.unedo* applications, since extended the existing literature, among others, by working with plants from the Moroccan geographical origin. Considering that the need for innovative and smart technological solutions is on-demand and the future of food industry would largely depend on bioactive-systems and natural-sources of active compounds for food preservation, futures perspectives would also consider to evaluate the efficacy of these active solutions in terms of economic and industrial feasibility, safety, and environmental impact. Furthermore, the sensory impact of this emerging packaging-strategy should also be evaluated, to ensure that the final quality of these packaged-products would not be compromised.

CRedit authorship contribution statement

Nisserine El Hammadi: Writing – original draft, Methodology, Investigation, Formal analysis, Data curation. **María Pilar Almajano:** Writing – review & editing, Visualization, Supervision, Resources, Methodology, Funding acquisition, Conceptualization. **María Vicenta Pastor:** Methodology. **Idoia Codina-Torrella:** Writing – review & editing, Visualization, Validation, Supervision, Methodology, Investigation, Data curation.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.lwt.2024.117110>.

Data availability

No data was used for the research described in the article.

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Glossary

- ABTS**: 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid)
- ANOVA**: Analysis of Variance
- AULE**: *Arbutus unedo* L. leaves extract
- AULE40**: *Arbutus unedo* L. leaves extract (obtained with a solution of 40:60 ethanol:water)
- AULE60**: *Arbutus unedo* L. leaves extract (obtained with a solution of 60:40 ethanol:water)
- AULE80**: *Arbutus unedo* L. leaves extract (obtained with a solution of 80:20 ethanol:water)
- CaCl₂**: Calcium Chloride.
- CR**: Crosslinking solution
- DAD**: Diode-Array Detector
- EtOH**: Ethanol
- GAE**: Gallic Acid Equivalent
- HPLC**: High-Performance Liquid Chromatography
- MeLo**: Methyl Linoleate
- PBS**: Phosphate Buffered Saline.
- PV**: Peroxide Value
- O/W**: Oil-in-water emulsion
- QE**: Quercetin Equivalent
- RT**: Retention Time.
- SEM**: Scanning Electron Microscopy
- TBARS**: Thiobarbituric Acid Reactive Substances
- TFC**: Total Flavonoid Content
- TPC**: Total Phenolic Content
- UV-vis**: Ultraviolet–visible Spectroscopy