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Flavonoid- and limonoid-rich extracts from lemon pomace by-products: Technological properties for the formulation of o/w emulsions

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ABSTRACT

Lemon pomace, the by-product derived from the industrial processing of citrus fruits for juice production, is a matrix rich in flavonoids and limonoids, bioactive compounds with interesting functional properties. The aim of the present work was to investigate the technological properties of flavonoids and limonoids-rich extracts recovered from lemon pomace by-products in model oil-in-water (o/w) emulsions. Two extracts, obtained with different extraction procedures, were characterized in terms of surface properties, either as pure extract or in combination with pea protein isolate. Then, the most surface-active extract was used to enrich, at increasing concentrations (0.5%, 1% and 2%), o/w emulsions (20% w/w) stabilized by pea proteins. Droplet size distributions, microstructure, flow behavior and oxidative stability were investigated. The extracts were able to lower the surface tension of the protein interfacial layer, with a concentration dependent behavior. In emulsions, depending on the amount added, the extract significantly altered the dispersion degree, by increasing oil droplet size and inducing flocculation at the highest extract to pea protein ratios. The emulsions showed a Newtonian behavior, with the exception of the sample with the highest amount of extract (2%) which also presented a yield stress. In accelerated oxidation tests, the extracts were shown to improve the chemical stability of the emulsions; however, the physical stability was impaired in the systems with the highest extract amounts (1%, 2%). Limonoids- and flavonoids-rich extracts recovered from lemon pomace can be considered promising as multifunctional ingredients in o/w emulsions, provided that their effect on the colloidal properties is properly addressed.

1. Introduction

The plant-based food processing sector is currently under expansion and, with it, the amounts of generated by-products. Hence, improved waste management is essential to mitigate the negative environmental impacts of fruit and vegetable processing industries (Satari & Karimi, 2018). The reuse of agro-industrial by-products can represent a renewable source for some food additives or even originate new added-value ingredients with functional properties, which will benefit the entire food system (European commission, 2015). For instance, by-products can contain polysaccharides, organic acids, proteins, and other compounds, which make them a rich source of natural compounds that can potentially be applied in the food industry as food ingredients or additives sources (Szabo et al., 2018). Furthermore, some of these natural compounds may also be regarded as nutraceutical ingredients or complements, allowing for the development of products with enhanced nutritional value, potential health benefits, longer shelf-life, as well as improved sensory profile (Abuajah et al., 2015; Carocho et al., 2015).

Italy represents the second nation after Spain in citrus production, with 2,635,000 tons produced per year (ISMEA, 2019). The industrial processing of citrus fruits for juice production generates citrus pomace, a by-product made of peels, squeezed pulp residues, seeds and residual fruits (Mahato et al., 2018). From a chemical viewpoint, it is composed of carbohydrates, organic acids (mainly citric and malic acids), lipids, mineral elements (mainly nitrogen, calcium and potassium), volatile components (e.g. alcohols, aldehydes, ketones, esters and hydrocarbons), enzymes, pigments and vitamins (Tamburino & Zema, 2009; Valenti et al., 2016). Furthermore, lemon pomace is a rich source of

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bioactive compounds like flavonoids and limonoids, such as eriocitrin, narirutin and hesperidin, for the flavonoids fraction, and limonin, nomilin and obacunone for the limonoids fraction (Russo et al., 2016). Both flavonoids and limonoids have been proved to exert interesting beneficial properties, including antioxidant, anti-inflammatory, anti--allergic, antiviral, antiproliferative, antimutagenic, and anti-carcinogenic effects (Wedamulla et al., 2022).

Considering the rapid growth of the functional food sector, the exploitation of citrus by-products as a source of functional compounds and their application into foods could be promising. Some authors have explored the possibility of using a flour obtained from Bergamot pomace in shortbread biscuits to replace wheat flour and improve the nutritional properties of the product (Laganà et al., 2022). In another work, a bioactive-rich extract obtained from lemon pomace was used to produce functionalized biscuits that showed improved phenolic content and antioxidant activity as well as a prolonged resistance to lipid oxidation. Moreover, despite the increased bitter perception due to specific flavanone and limonoid compounds deriving from the extract, sensory properties were considered acceptable by consumers (Imeneo et al., 2021), paving the way to the concrete exploitation of such bioactive-rich extracts in real matrices.

Bioactive-rich extracts recovered from vegetable by-products can also exert relevant technological functionalities that may help in the formulation of emulsified and gelled systems. An example can be found in phenolic-rich extracts derived from olive leaves that were able to lower the surface tension and exert emulsifying properties both in model and real systems (Flamminii et al., 2020). Similarly, hydroalcoholic extracts rich in polyphenols, proteins and saponins, obtained from argan shell (*Argania spinosa* L.) by-products, proved capable of reducing interfacial tension at the soybean oil/water interface and stabilizing o/w emulsions (Bouhoute et al., 2020). To date, however, the information available in literature on the potential technological properties in emulsified systems of flavonoids and limonoids recovered from lemon pomace is scarce. The purpose of this work was thus to investigate the technological properties of extracts obtained from citrus processing by-products, rich in limonoids and flavonoids, and their exploitation in emulsified model systems in combination with a pea protein isolate chosen as emulsifier. Two extracts, obtained from lemon pomace with different extraction procedures, were characterized in terms of content of flavonoids and limonoids and antioxidant activity. The surface properties, either as pure extracts or in combination with pea proteins, were also evaluated. Then, the most surface-active extract was used to enrich, at increasing concentrations (0.5%, 1% and 2%), o/w emulsions (20% w/w) stabilized by pea proteins. Droplet size distributions, microstructure, flow behavior, physical and oxidative stability were investigated.

2. Materials and methods

2.1. Materials

The extracts of lemon by-products (pomace) used in this study were provided by the Agricultural Department of the Mediterranean University of Reggio Calabria (IT). The extraction procedure was performed following the methods reported by Imeneo et al. (2022). An ethanol: water mixture (1:1) was used as extraction solvent with a solvent:sample ratio of 5:1 (v/w). Two extraction conditions were applied: conventional extraction (maceration) for 30 min at 70 °C to produce the PAE70 (Pomace Antioxidant Extract) and ultrasound assisted extraction (ω: 50%, pulsation time on 1s off 1s) for 30 min at 70 °C using a Sonoplus Ultrasonic Homogenizer (Series 2000.2, HD 2200.2,BANDELIN, Ultraschall seit 1955, equipped with a VS 70T probe of 13 mm), used to obtain the UPAE70 (Ultrasound Pomace Antioxidant Extract).

The pea protein isolate (PPI) (Pisane™ C9), produced by Cosucra Groupe Warcoing S.A. (Warcoing, Belgium), was kindly donated by VICTA Food SRL (Mogliano Venet, Italy); its composition, as reported in the technical sheet, was: 81.7% protein, 9% fat, 5% salt, 1.4% dietary fiber, 0.5% carbohydrates. Sunflower oil was purchased from a local store. All the reagents used were of analytical grade.

2.2. Antioxidant characterization of lemon by-product extracts

Total phenolic content (TPC), total flavonoid content (TFC), antioxidant activity determinations (DPPH and ABTS) were carried out following the same methods reported by Imeneo et al. (2022). Individual phenolic content and limonoid determinations were performed using the method reported by Gattuso et al. (2023). A UHPLC-DAD (Knauer, Berlin, Germany) system equipped with a Kinetex 2,6 μm Biphenyl 100 Å (Phenomenex®, Torrance, CA, USA) was used for chromatographic separation; the injection volume was 5 μL. The elution conditions used for flavonoid and limonoid separation are reported in Supplementary Table S1. For the quantification of phenolic compounds (ferulic Acid; eriocitrin; narirutin; naringin and hesperidin) and limonoids (limonin, nomilin and obacunone) external standards (Merck, Darmstadt, Germany) at a concentration between 1 and 100 mg L^{-1} were used. Each compound was quantified at a wavelength of 280 nm and the results were expressed as mg L^{-1}.

2.3. Technological characterization of citrus by-products extracts

2.3.1. Surface tension

Air/water surface tension of lemon by-products extracts was evaluated alone and in combination with pea protein by means of a tensiometer (Sigma 700/701, Attention, Biolin Scientific, Espoo, Finland) equipped with a platinum Wilhelm plate. Different extract solutions (0.001%–2% v/v) were prepared in 50 mM phosphate buffer at pH 6. The ethanol contribution to the surface tension of the diluted systems was negligible. As regards the mixed extracts and pea protein suspensions, a fixed concentration of the pea protein isolate (0.2% w/v) was combined with different concentrations of citrus extract (0.1%-1%–2% v/v). Measurements were carried out at 25 °C after an equilibration time of 30 min.

2.3.2. Emulsions preparation

Oil-in-water model emulsions stabilized with PAE extract as sole emulsifier were prepared by mixing 5% (w/w) sunflower oil with the aqueous phase consisting of different concentration of PAE70 extract (0.005%; 0.01%; 0.05%; 0.1%; 0.5%; 1% v/v) in phosphate buffer solution (50 mM, pH 6). Oil-in-water model emulsions stabilized with the mix PAE70 extract/pea protein isolate were prepared as follow: a 5% (w/v) stock solution of PPI was prepared in ultrapure water and left to hydrate overnight. Sunflower oil (20% w/w) was mixed with the aqueous phase composed of a fixed concentration of PPI (1% w/v); different concentrations of PAE70 extract (0.5%, 1% and 2% v/v) were added to obtain the enriched samples (PAE0.5, PAE1 and PAE2, respectively). Emulsions with no added PAE70 were taken as reference (CON). Emulsions were obtained in a two-step homogenization process: first a pre-emulsion was created with a rotor-stator device (YellowLine DI 25 Basic, IKA Werke GmbH & Co, Germany) set at a speed of 13,500 rpm for 60 s and then homogenized with a high-pressure homogenizer (Panda plus 2000, GEA Niro Soavi, Parma, Italy) set at 150 bars for 10 cycles.

2.3.3. Particle size distribution

The particle size and distribution of oil-in-water model emulsions were analyzed by static light scattering (Mastersizer 3000, Malvern Instruments Ltd, Worcestershire, UK), following the method by Flamminii et al. (2023). Emulsions were added to the dispersion unit (Hydro 2000S, Malvern Instruments Ltd., Worcestershire, UK) in distilled water at 2000 rpm up to reach an obscuration between 5% and 6%, as evaluated with preliminary tests. For sunflower oil, a particle refractive index of 1.474 with a particle adsorption of 0.01 was chosen, while for

water a refractive index of 1.330 was used. Droplet size measurements are reported as the volume-weighted mean diameter $D_{4,3}$ and particle size distribution. Flocculated state of oil droplets was obtained, diluting the emulsions in a 1% (w/v) SDS solution, as described by D'Alessio et al. (2022). The Flocculation Index % (FI%) was then calculated as follows (Equation (1)):

$$FI\% = (D4,3_{H2O} / D4,3_{SDS} - 1) \times 100 \quad (1)$$

2.3.4. Flow behavior

The flow curves of the emulsions were obtained by a controlled stress-strain rheometer (MCR 302, Anton Paar, Graz, Austria) using a concentric cylinder geometry and temperature control water bath set at 20 °C. Flow curves were measured by recording the shear stress at increasing shear rates from 0.3 to 230 s^{-1}. They were described using the Herschel–Bulkley model (Equation (2)):

$$\sigma = \sigma_0 + k\,\dot{\gamma}^n \quad (2)$$

where σ is the shear stress (Pa); σ_0 is the initial shear stress (Pa); k is the consistency index (Pa*s); γ is shear rate (s^{-1}), and n is the flow behavior index (dimensionless) (Vélez-Erazo et al., 2020).

2.3.5. Microstructure

The microstructure of the emulsions was observed using a Nikon A1R Microscope (Nikon Corporation, Tokyo, Japan), controlled by the Nikon NIS Elements software ver. 4.40, equipped with a Plan Apo lambda 40 × oil objective and visualized by Transmitted light Detector (TD). Microstructure images were acquired with a Nikon DS QiMc U3 camera (Nikon Corp., Tokyo, Japan).

2.3.6. Creaming stability

The creaming stability of the emulsions was determined using a modified version of the procedure developed by Keowmaneechai and McClements (2002). Ten grams of each emulsion was placed in a glass tube and stored at a temperature of 22 °C for 7 days. During the storage period, separation occurred, resulting in the formation of a cream layer at the top and a liquid serum layer at the bottom. The height of the entire emulsion (Hc) and the height of the serum layer (Hs) were measured. The degree of creaming was quantified as a creaming index (%) using the following Equation (3):

$$CI = Hs/Hc \times 100 \quad (3)$$

2.3.7. Oxidative stability

The oxidative stability of the samples was tested using the OXITEST Device (Velp Scientifica, Usmate, MB, Italy). Ten grams of o/w emulsion were weighed into the sample cells with a uniform distribution. The device temperature was set at 90 °C and the oxygen pressure was set at 6 bar (Katsouli et al., 2017). The oxidative stability values of the samples were determined by the Oxisoft™ software and reported as induction period (IP) value, corresponding to the absolute oxygen pressure drop inside the instrument chambers, due to the oxidation reaction, automatically calculated from the oxidation curve by the graphical method, based on two tangent methods.

2.3.8. Statistical analysis

Statistical analyses were performed using Origin (Pro) Version number 2022 (OriginLab Corporation, Northampton, MA, USA). Data were analyzed by one-way ANOVA, and analyses of differences between experimental groups were made using Tukey's multiple-comparison tests with a significance threshold of 0.05.

3. Results and discussion

3.1. Chemical-functional characterization of lemon by-product extracts

The content of total phenolic compounds, total flavonoids and individual flavonoids and limonoids detected in the lemon pomace extracts are reported in Table 1, along with the antioxidant properties evaluated by DPPH and ABTS assays. By the adoption of two different extraction procedures, extracts with significantly different chemical composition and properties were obtained. Indeed, PAE70, obtained with conventional extraction conditions (30 min, 70 °C), was characterized by significantly higher values, compared to UPAE70 (obtained through the ultrasound assisted extraction), in terms of TPC, TFC, ABTS, as well as in terms of individual antioxidant compounds.

Such results are consistent with other works present in literature which showed that, other parameters being equal, conventional maceration was more effective in recovering bioactive compounds from citrus by-products, when compared to microwave and ultrasound assisted procedures (Gattuso et al., 2023). During the ultrasound-assisted extraction, the application of high extraction temperatures can lead to the fragmentation of cell walls by boosting the activity of several enzymes, therefore facilitating cell wall damage and enhancing the possible extraction of bioactive compounds (Dahmoune et al., 2013; Ma et al., 2016); however, a certain quantity of polyphenols may be degraded due to some process variables, such as extraction time and temperature, ultrasound power, and other parameters (Papoutsis et al., 2018). In addition, dried lemon residues were found to be resistant to ultrasonic energy, however showing cell wall damage at the end of the ultrasonic extraction process. Furthermore, the lower value of total polyphenols in UPAE70 could be attributed to the reaction of some flavonoids with the free radicals produced in the extraction solution due to the power of ultrasound, as flavonoids could behave as hydrogen donors and singlet oxygen quenchers thanks to their high redox potential (Ignat et al., 2011; Riesz et al., 1985; Rappoport, 2004). Consequently, the lower antioxidant capacity of UPAE70 could be related to the lower content of total polyphenols and flavonoids.

As far as flavonoid content is concerned, eriocitrin and hesperidin were the most abundant compounds in both the extracts (645.04 ± 11.68 and 442.11 ± 22.36 mg L^{-1}, respectively) while for citrus limonoids, polycyclic secondary metabolites, located mostly in seeds, fruits and peel tissues of citrus fruits (Gualdani et al., 2016), the most representative compounds found in the lemon pomace extracts were limonin, nomilin and, to a lesser amount, obacunone. Examples of chromatographic profiles of individual flavonoids and limonoids are provided in Supplementary materials (Figs. S1 and S2).

In general, the values reported for TPC, TFC, ABTS and DPPH, as well

Table 1
Total phenolic and flavonoid contents (TPC and TF), antioxidant properties (ABTS and DPPH) and individual flavonoid and limonoid contents of the two lemon pomace extracts. Abbreviation: ns:not significant; **:significant at $p < 0.05$

Samples	PAE70	UPAE70	Significance
TPC (mg GAE mL^{-1})	1.47 ± 0.08	1.18 ± 0.04	**
TF (mg CE mL^{-1})	0.47 ± 0.02	0.33 ± 0.02	**
ABTS (μM TE mL^{-1})	3.99 ± 0.12	3.49 ± 0.11	*
DPPH (μM TE mL^{-1})	1.70 ± 0.06	1.57 ± 0.08	ns
Flavonoids [mg L^{-1}]			
Ferulic Acid	25.45 ± 1.36	21.24 ± 0.98	*
Eriocitrin	645.04 ± 11.68	549.14 ± 14.23	**
Narirutin	16.24 ± 0.78	16.55 ± 1.41	ns
Naringin	36.73 ± 1.64	28.15 ± 0.68	**
Hesperidin	442.11 ± 22.36	201.15 ± 15.26	**
Limonoids [mg L^{-1}]			
Limonin	70.50 ± 1.36	46.27 ± 1.11	**
Nomilin	29.44 ± 0.74	22.64 ± 0.26	**
Obacunone	2.47 ± 0.10	0.68 ± 0.04	**

as for the content of individual flavonoids and limonoids can be considered in line with results present in literature on the same matrices (Imeneo et al., 2021, 2022), provided that the differences in the expression of the results are taken into account (wet basis/dry basis).

3.2. Surface properties of lemon pomace extracts

The adsorption properties of the lemon pomace extracts were characterized at the air/water interface, which was used as a rough estimation of the interfacial behavior at the oil/water interface. In Fig. 1 the air/water surface tension of PAE70 and UPAE70 buffered solutions is depicted as a function of concentration. In the concentration range between 0.001% and 0.1%, a decrease of the surface tension compared to pure water was observed, even though with no concentration dependence. Testing concentrations higher that 0.1%, a sharp decrease was experienced for both the extracts, exhibiting a dose-dependent interfacial activity followed by a plateau region with concentrations higher than 1%, indicating a saturation at the air/water interface with values of about 31.06 mN/m, similar to those reported for low molecular weight surfactants (Bezelgues et al., 2008). Despite the difference of the extracts in terms of limonoid and flavonoid composition and content, related to the two extraction procedures adopted, no significant differences were observed in their overall surface properties. Among all the compounds present in the extract, flavanone glycosides like eriocitrin and hesperidin may display interfacial activity due to their chemical structure characterized by a hydrophilic portion, represented by the saccharide group, and a hydrophobic unit, represented by the flavonoid backbone (Caballero et al., 2022). Indeed, hesperidin exhibited interfacial activity, improving the stability of a chitosan-stabilized o/w emulsion (Dammak et al., 2019), and enhanced gluten capacity to absorb on water/oil interface and hence its emulsifying capacity (Jiang et al., 2022). Interfacial properties were demonstrated also for naringin, which showed good emulsification activity (Luo et al., 2011). In terms of surface properties, it is important to point out that the contribution of other amphiphilic compounds such as proteins and polysaccharides, probably co-extracted but not quantified in the present work, is not to be excluded.

Considering such promising results and the highest content of flavonoids and limonoids, PAE was chosen for further characterization in mixed systems made of extracts and pea protein isolate. Fig. 2 shows the surface tension value of mixed systems prepared by keeping constant the amount of PPI (0.2% w/v) and adding increasing concentrations of PAE (0.1%, 1% and 2% v/v), together with the pure extracts with no proteins

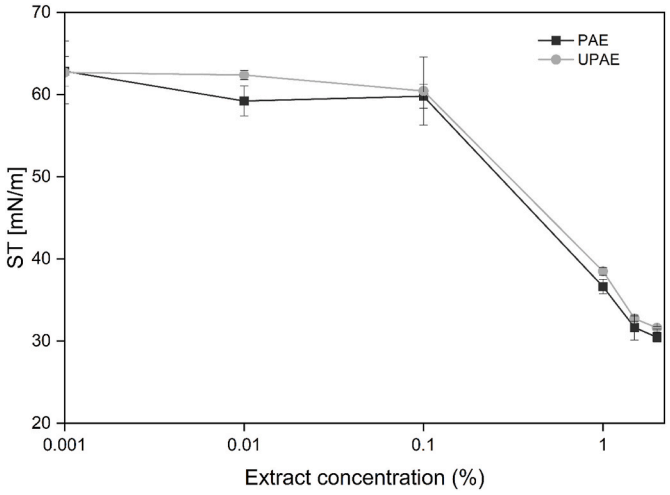

Fig. 1. Extract surface tension at the air/water interface under buffered condition (phosphate buffer, 50 mM, pH 6). The surface tension value of water under the same conditions was 72.00 ± 0.05 mN/m.

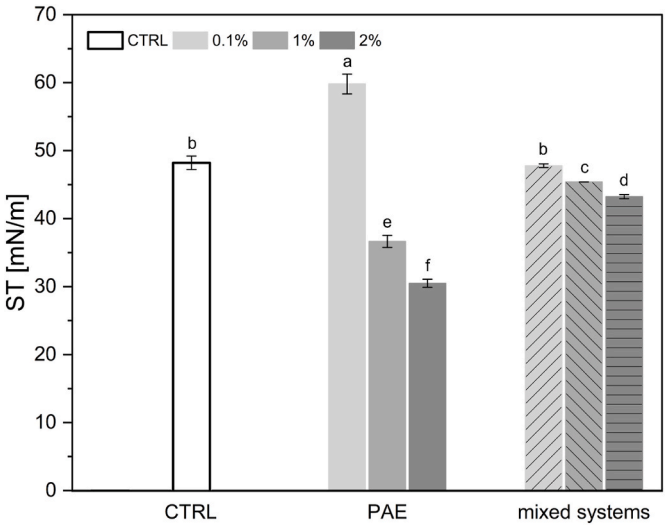

Fig. 2. Surface tension of CTRL (PPI 0.2% w/v), PAE (0.1% – 1% – 2% v/v) and mixed systems of PPI (constant at 0.2% w/v) with the addition of different amounts of PAE (0.1% – 1% – 2% v/v). The surface tension was analyzed under buffered conditions (phosphate buffer, 50 mM, pH 6).

added (PAE) and the control made of protein only at the same concentration (CTRL). The concentration of proteins used in the mixed systems was selected by evaluating the adsorption behavior of PPI over increasing concentrations, according to which at 0.2% (w/v) a full coverage of the interface was achieved (data not shown).

When PAE was added to PPI, no detectable effects were observed for the lowest concentration tested (0.1%) while a significant decrease of surface tension occurred at 1% and 2% PAE, to an extent that was dependent on the amount of extract added to the mixture.

However, the decrease of surface tension was not as effective as the pure extract systems themselves analyzed at the highest concentrations, meaning that in the mixed systems the air/water interface was mainly dominated by the pea proteins, but the addition of flavonoids and limonoids was able to further lower the free energy of the interface. This behavior may be due to different mechanisms, a co-adsorption at the interface of the pea proteins and the extract or a possible interaction between bioactive molecules and pea proteins with a general enhancement of the adsorption behavior of both the compounds. Indeed, non-covalent protein-polyphenol complexes increased protein flexibility, solubility, and surface hydrophobicity, thus improving the ability of protein to adsorb on oil-water surfaces (Li et al., 2020). Further investigations are underway to understand the interaction of pea proteins and such bioactive compounds at the air/water interface.

3.3. Emulsifying capacity

The emulsifying capacity of lemon pomace extract was evaluated by testing the ability to create and stabilize oil-in-water (o/w) emulsions. In a first attempt, in order to test the emulsifying ability of the pure extract, no other emulsifiers were added to the continuous phase and thus o/w model emulsions were formulated by mixing 5% (w/w) sunflower oil with an aqueous phase containing different concentrations of the sole PAE extract (0.005%; 0.01%; 0.05%; 0.1%; 0.5%; 1% v/v, buffered media at pH 6). Interestingly, emulsions prepared exclusively with PAE were not stable regardless of the concentration used and of the homogenization conditions adopted. These results indicate that although flavonoids and limonoids could diffuse to the oil/water interface, they provided a weak stabilization to the emulsions likely because they form a very thin interface with limited steric hindrance that promotes quick merging of oil droplets through film rupture with a consequent phase separation. The destabilization phenomena can be also attributed to the

tendency shown by some citrus flavonoids to migrate towards the aqueous phase and undergo crystallization, especially when oil with long triglyceride chains is used as dispersed phase (Caballero et al., 2022).

Such results draw attention to the fact that lemon by-product extracts, rich of flavonoids and limonoids, may be used in combination with other emulsifiers to achieve an adequate stabilization of the oil/water interface.

Pea protein isolate (1% w/v) was thus used to stabilize 20% (w/w) oil-in-water emulsions added with increasing PAE concentrations (0.5%, 1% and 2% v/v); emulsions with no PAE added were used as reference. Pea proteins were chosen as they are currently of great interest for the formulation of innovative plant-based food products thanks to their low cost, high sustainability and technological functionality (D'Alessio et al., 2022; Pam Ismail et al., 2020). In the emulsions added with PAE (Fig. 3A), a broadening of the droplet size distribution was verified, to an extent that was dependent on the amount added: the more the PAE, the wider the droplet size distribution. Besides the effect on the population width, it can be seen that there is a clear shift from monomodal distribution with a limited tail on the right (CON), towards polydisperse systems in which several populations are present, centered either on small and big droplet sizes (PAE2). As far as the droplet size is concerned, the $D_{4,3}$ passed from 2.13 ± 0.60 μm in the CON sample to 4.18 ± 1.52 μm, 10.45 ± 2.16 μm and 33.38 ± 2.81 μm in PAE05, PAE1 and PAE2, respectively. When the same samples were measured in a SDS solution to eliminate droplet aggregation phenomena (Fig. 3A'), no broadening in the droplet size distribution was verified, while at the highest extract concentration (PAE2) a bimodal distribution was observed, with a prevalence of large droplets, along with a slight shift in droplet size (from 1.49 ± 0.17 to 2.72 ± 0.44 μm). This means that the polydispersity and the broadening of the distribution were due to flocculation which in turn was caused by the addition of PAE, as also confirmed by the flocculation index (from 43% of the CON to 173%, 528% and 1151% of PAE05, PAE1 and PAE2, respectively).

Since PAE addition also caused an acidification of the continuous phase (from pH 7.5 in CON to pH 6.2 in PAE2), in another set of experiments, after the extract enrichment, the pH of the dispersed phase was corrected to the pH value of the control and used for emulsification (Fig. 3B). In this case too, a broadening of the distribution occurred, together with the appearance of a polymodal population with a prevalence of larger droplets, especially in PAE2. Testing the emulsions with SDS, reducing therefore the contribution of flocculation phenomena (Fig. 3B'), the addition of the extract caused limited variations in the samples. Depending on the amount added, PAE could then alter the dispersion degree of the samples, and induce flocculation phenomena which, however, were more evident when the pH of the systems decreased due to PAE addition. The effect of pH on the occurrence of flocculation in PPI emulsions has already been documented in literature; indeed, the formation of flocs was observed in a wide range of pH values (3, 5, 7 and 9), with a more pronounced effect under pH 5 and pH 7 (Liang & Tang, 2013). Similar results were observed in emulsions stabilized with whey protein isolate (WPI) with the addition of naringin, in which the direct influence of pH on droplet size was evaluated. The results showed that at pH 4, the size of the droplets increased with increasing concentrations of naringin, while this phenomenon did not occur at neutral or alkaline pH levels. Naringin addition thus caused the destabilization of the emulsion, albeit only when its addition was combined with a pH value close to the isoelectric point of the proteins

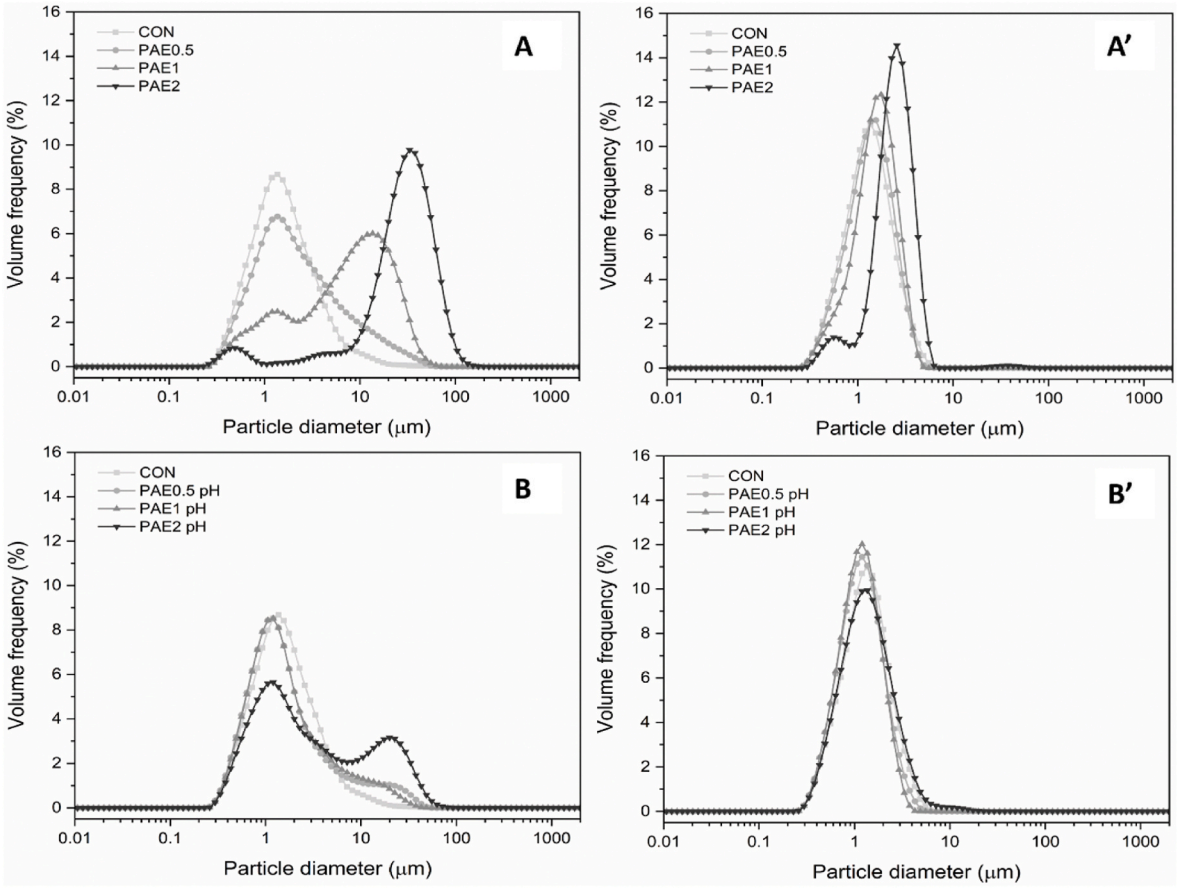

Fig. 3. Particle size distribution of the emulsions stabilized by 1% PPI (CON) with the addition of PAE extract at 0.5%, 1% and 2% concentrations (PAE0.5, PAE1, PAE2, respectively). In A and A′, the pH of the enriched systems was not corrected while in B and B′ the pH of the enriched systems was corrected to CON value. Measurements in A′ and B′ were carried out in a 1% SDS solution.

(Hu et al., 2020). Besides this, it is clear from Fig. 3B that flocculation also occurred as a consequence of the mere addition of PAE flavonoids and limonoids. Two hypotheses may be considered: PAE may either have induced electrostatic interaction among the droplets which promoted the formation of agglomerates or have affected the physical properties of PPI interfacial layers. It must indeed be recalled that the surface properties of PPI interfacial layers were affected by PAE addition; further investigations are needed to unravel the factors and mechanisms underpinning flocculation phenomena in PPI emulsions as induced by PAE enrichment.

3.4. Microstructure

The microstructure of the emulsions was studied through optical microscopy (Fig. 4). CON and PAE0.5 samples (Fig. 4A and B) were characterized by a fine and homogeneous dispersion of oil droplets. However, starting from PAE1 (Fig. 4C), a slight agglomeration of particles can be observed, clearly visible in PAE2 (Fig. 4D) with large and separated clusters of particles, together with the appearance of larger particles. The microstructure is therefore consistent with the results of particle size distributions (Fig. 3A and A') as previously observed. As the concentration of the extract in the emulsion increased, larger droplet sizes were noticed together with flocculation phenomena.

3.5. Flow behavior

In Fig. 5 the flow curves of the different emulsions are depicted. CON and PAE0.5 samples showed an overlapped trend with a Newtonian behavior. The addition of higher amounts of PAE (PAE1 and PAE2) caused significant variations ($p < 0.01$) in the flow curves particularly evident in PAE2, in which the appearance of a yield stress was observed.

The parameters of each emulsion fitted with the model of Herschel-Bulkley and apparent viscosity at 170 s^{-1} of shear rate are shown in Table 2. No significant differences were found in CON and PAE0.5 parameters, likely as a consequence of their similar dispersion degree and microstructure. Increasing the amount of extract caused a reduction of the flow index which passed from a Newtonian behavior in CON and PAE0.5 to a shear thinning behavior in PAE 1 and PAE2. In this last case, a yield stress appeared, and the apparent viscosity and consistent index resulted significantly higher compared to the other samples. The results confirm that the addition of the highest amount of lemon pomace extract to the emulsions led to flocculation, causing an increase in viscosity and a change in flow behavior as a result of the entrapment of the continuous phase within the flocs (McClements, 2004).

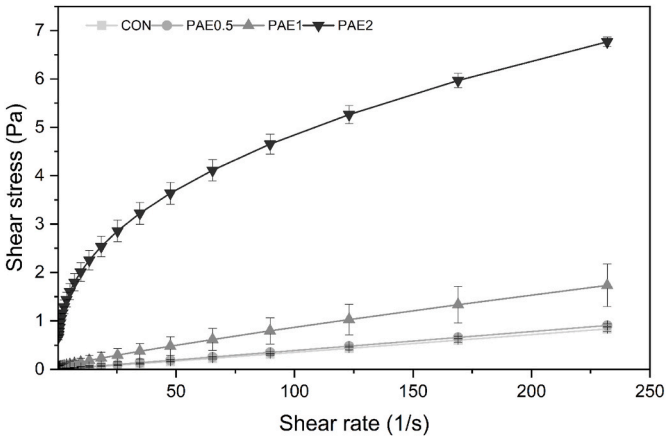

Fig. 5. Flow curves of emulsions at increasing shear rates from 0.3 to 230 s^{-1}.

Table 2
Rheological parameters (σ0, k, and n) of Herschel-Bulkley model to flow curves of the emulsions, and apparent viscosity at a shear rate of 170 s^{-1}.

	CON	PAE0.5	PAE1	PAE2
k	0.003 ± 2.28E-04 [a]	0.003 ± 1.18E-04 [a]	0.018 ± 0.012 [a]	0.7 ± 0.16 [b]
n	1.03 ± 0.008 [a]	1.01 ± 0.004 [a]	0.86 ± 0.084 [b]	0.41 ± 0.038 [c]
σ_0	0.023 ± 8.1E04 [a]	0.007 ± 0.009 [a]	0.028 ± 0.026 [a]	0.23 ± 0.07 [b]
$\eta_{(170)}$ (mPa.s)	3.58 ± 2.22E-01 [a]	3.9 ± 1.54E-01 [a]	7.9 ± 2.2 [b]	35.04 ± 6.46E-01 [c]
R^2	0.999 ± 1.56E-0.5	0.999 ± 4.65E-05	0.999 ± 2.24E-05	0.997 ± 2.13E-03

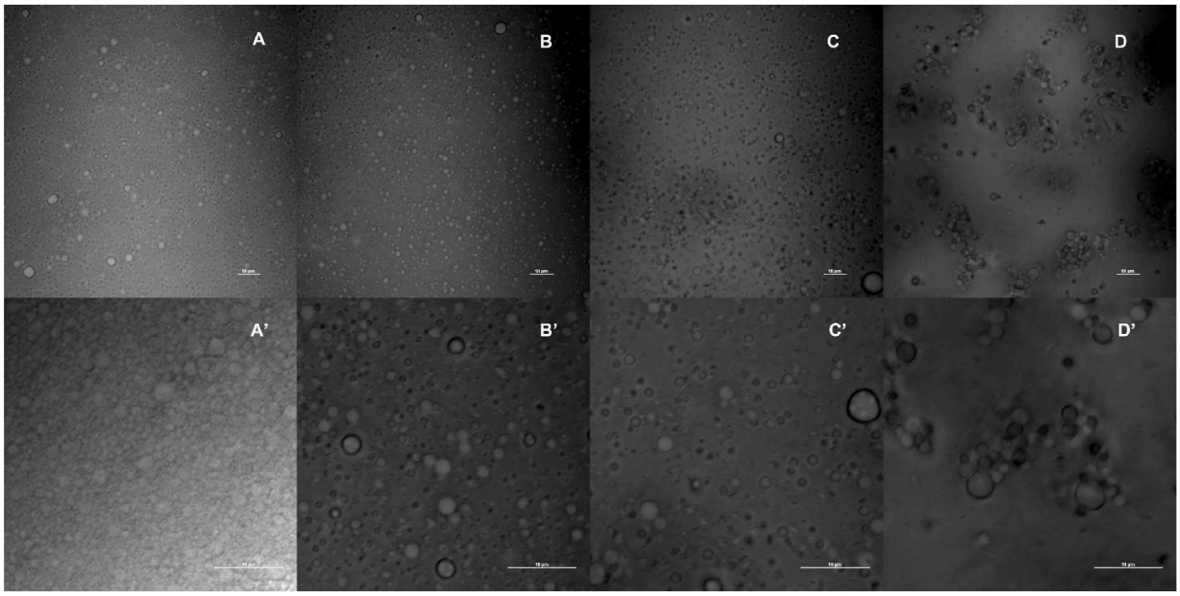

Fig. 4. Images of the emulsions obtained with PPI and PAE extract at increasing concentration; CON (A; A′), PAE0.5 (B; B′), PAE1 (C; C′), PAE2 (D; D′); A′, B′, C′, D′ are images obtained by digital zoom.

3.6. Effect of extract enrichment on physical and oxidative stability

The physical stability of the emulsions was evaluated by means of the creaming index (CI), a parameter commonly used to monitor the physical stability of multiphasic systems under quiescent storage. The CI value of all the samples increased during the 7 days of storage (Fig. 6), but with different rates; after 1 day of storage, the CI rate followed the order CON < PAE0.5 < PAE1 < PAE2. This last sample experienced a very steep increase of CI which, on the second day, settled at a percentage of 22% ± 2% and remained almost stable (24% ± 1%) over the entire storage. The same was observed in PAE1, which showed an increase after three days of storage, reaching values comparable to PAE2 (24%–26% ± 1%). Conversely, CON and PAE0.5 presented a very similar trend over the storage reaching values of 10%–12% ± 1%. Thus, the enrichment with PAE at higher concentrations (PAE1 and PAE2) caused an impairment of the emulsified systems which underwent physical destabilization more rapidly and to a higher extent compared to CON and PAE0.5 emulsions ($p < 0.05$). This result may be related to several factors among which a key role was played by the effect of PAE on PPI emulsifying capacity. Additionally, these results may also be explained by the higher degree of flocculation observed in PAE1 and PAE2 samples, which led to larger particle size in the emulsions and, consequently, faster creaming (McClements & Jafari, 2018).

As a last step, the emulsion samples were submitted to an accelerated oxidation test by means of OXITEST, an effective method useful to evaluate the resistance to oxidation of a matrix and the eventual protective effect of bioactive ingredients within the extract. The induction period (IP) value, i.e., the time before the onset of lipid oxidation, is shown in Fig. 7. Under the test conditions, the extract was found to enhance the chemical stability of the emulsions with a dose dependent effect. However, the effect was significant only in the PAE2 sample when compared to the control (Fig. 7), confirming the *in vitro* antioxidant activity shown by the extract (Table 2). The lemon pomace extract could thus also exert antioxidative properties in o/w emulsified systems, with an effectiveness which is dependent on the amount added. Such results are in agreement with previous findings on pure flavonoids: naringin, adsorbed at the interface of oil particles in a whey protein isolate-stabilized emulsion, displayed both interfacial properties and antioxidant activity by reducing the release of hydroperoxides from corn oil (Hu et al., 2020). In another work, a nano-encapsulated phenolic-rich extract obtained from orange peel, produced via double-emulsion technology, was effective in retarding oxidative phenomena in edible oils (Rashid et al., 2022). An enhanced stability towards oxidation was also observed in whey protein stabilized o/w emulsions loaded with hesperidin, in a dose-dependent manner (Wang et al., 2023). However, it must be specified that, in the literature, works dealing with the effect of limonoids and flavonoids recovered from citrus by-products on the oxidative stability of emulsified model systems are very limited.

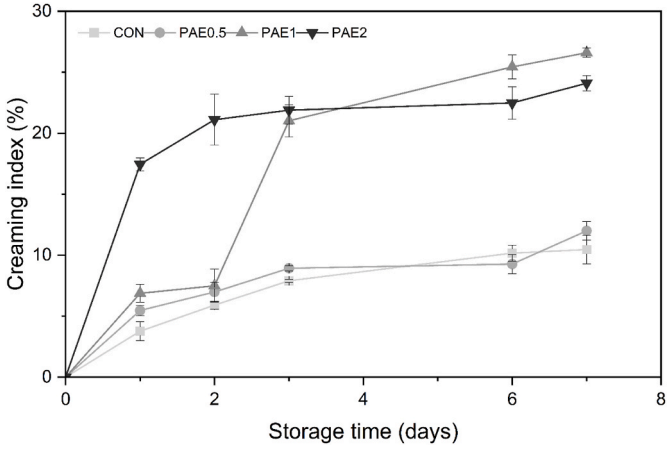

Fig. 6. Creaming index values (%) of the samples during 7 days of storage at 22 °C.

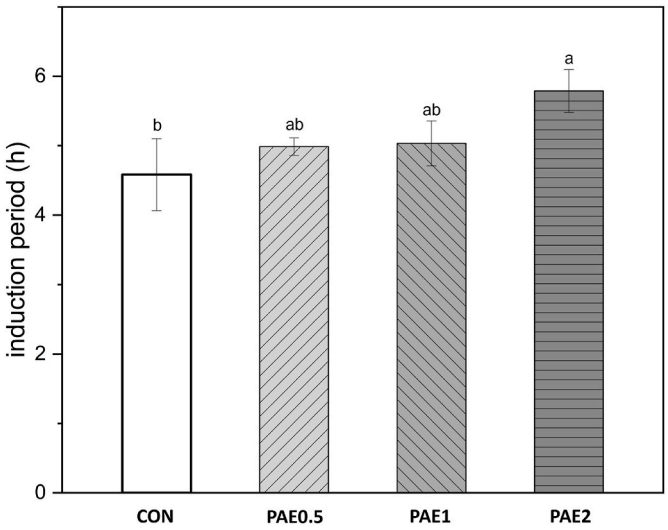

Fig. 7. Induction period values (h) of the samples obtained with the Oxitest method under accelerated conditions.

4. Conclusion

This study shows a possible and promising use of extracts recovered from lemon pomace by-products and rich in flavonoids and limonoids for the formulation of oil-in-water emulsions. Besides their potential health properties, such bioactive compounds were proven to be promising multifunctional ingredients for the shelf-life extension of o/w emulsions in terms of oxidative stability and for viscosity modulation. However, the use of lemon pomace extracts should be carefully considered as colloidal properties may be negatively affected, promoting a faster physical destabilization, the entity of which is only partly related to the decrease of the pH of the continuous phase due to extract enrichment. More investigations are actually ongoing to unravel the factors underpinning the interactions of lemon pomace extracts with PPI at the interface and their effects on emulsion properties and stability.

CRediT authorship contribution statement

Francesco Iervese: Writing – review & editing, Investigation, Formal analysis, Data curation. **Federica Flamminii:** Writing – review & editing, Writing – original draft, Methodology, Formal analysis, Conceptualization. **Giulia D'Alessio:** Writing – review & editing, Formal analysis, Data curation, Conceptualization. **Lilia Neri:** Resources, Formal analysis, Writing – review & editing. **Alessandra De Bruno:** Data curation, Formal analysis, Investigation, Writing – review & editing. **Valeria Imeneo:** Data curation, Formal analysis, Investigation, Writing – review & editing. **Luca Valbonetti:** Data curation, Formal analysis, Methodology. **Carla Daniela Di Mattia:** Writing – review & editing, Writing – original draft, Supervision, Resources, Project administration, Methodology, Formal analysis, Conceptualization.

Declaration of competing interest

1. All authors have participated in the design of the experiments, in the analysis of the data and in the drafting of the article.
2. This manuscript has not been submitted to, nor is under review at, another journal or other publishing venue.
3. The authors have no affiliation with any organization with a direct or indirect financial interest in the subject matter discussed in the manuscript

Data availability

Data will be made available on request.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.fbio.2024.104030.

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