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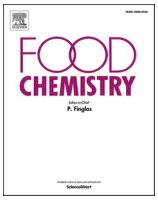

Lesser mealworm (*Alphitobius diaperinus*) protein concentrate conjugated with tannic acid improves the oxidative stability of $W_1/O/W_2$ emulsions loaded with beet by-product extract and linseed oil

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ABSTRACT

For improving the oxidative stability of a polyunsaturated oil, we *co*-encapsulated polyphenols from a concentrated beet by-product extract (CEB) with linseed oil using $W_1/O/W_2$ emulsions produced through emulsification with dynamic membranes of tunable pore size (DMTS), a low-energy high-throughput emulsification technology. Emulsions were stabilized with lesser mealworm protein concentrate (LMPC) and with an LMPC-derived antioxidant emulsifier (LMPC conjugated to tannic acid (LMPC-TA)). Regarding productivity, values of transmembrane flux were high (above 100 $m^3\ m^{-2}\ h^{-1}$), and of industrial interest. Regardless of the protein used, emulsions showed an encapsulation efficiency higher than 67.5 %, while droplet size ($D_{4,3}$) was below 8.28 μm. All emulsions were physically stable for 16 days at 4 °C, while at 25 °C, those stabilized with LMPC-TA had a less pronounced increase in $D_{4,3}$. In all cases, emulsions containing CEB and LMPC-TA inhibited oil oxidation, increasing the shelf life of the emulsions.

1. Introduction

Red beet (*Beta vulgaris* L.), the main vegetable grown worldwide, is attractive for human consumption due to its sweet flavor, nutritional attributes, and the technological applications of betalains as a natural colorant (de Oliveira et al., 2023). However, it is estimated that 40 % of this vegetable is not used, as the peels, leaves, and stems are considered waste (Camelo-Silva et al., 2024). Recent studies have reported that in addition to betalains, these discarded by-products are a source of phenolic acids (ferulic acid, caffeic acid, ellagic acid, vanillic acid, and sinapic acid), flavonoids (vitexin and quercetin), sinapaldehyde and resveratrol, which confer antioxidant, anti-inflammatory, chemopreventive properties, and improve intestinal health (de Oliveira et al., 2023; Lasta et al., 2019). However, ensuring the stability and bioavailability of these compounds is limited because they are susceptible to degradation under adverse conditions such as pH, light, oxygen, temperature, and hostile environments of the gastrointestinal system (enzymes and bile salts) (Arend et al., 2022; Mohammed et al., 2021). Regarding sensory properties, geosmin imparts an earthy flavor to foods, which is undesirable when adding beet extract (Shofinita et al., 2023). Therefore, approaches to preserve bioactivity and mask unwanted flavors of polyphenols present in concentrated beet by-product extract (CEB) are of industrial and scientific interest.

The production of multiple water-in-oil-in-water emulsions ($W_1/O/W_2$) through membrane emulsification is an interesting approach because it operates under mild process conditions and requires low energy consumption (Piacentini et al., 2014). A previous study proved that dynamic membranes of tunable pore size (DMTS) are suitable for producing $W_1/O/W_2$ emulsions that encapsulate bioactive compounds, reaching values of encapsulation efficiency higher than 72 % and droplet size below 7.4 μm (Wang et al., 2021a). Van der Zwan et al. (2008) were the first to report using DMTS to produce an emulsion of n-Hexadecane and water. Since then, this technique has been used in the preparation of food emulsions with lemon oil (Kaade et al., 2020; Kaade

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et al., 2023), carob pulp polyphenols (Wang et al., 2020), beet juice (Eisinaite et al., 2016), grape seed extract (Estévez et al., 2019; Wang et al., 2021a), and vegetable protein (Ettoumi et al., 2017). The DMTS system utilizes a bed of silica glass microspheres supported by a nickel microsieve. It is termed "dynamic" because the size of the microspheres and the bed height can be adjusted to modify the pore size and thickness of the microporous system (Kaade et al., 2020). This configuration makes the DMTS system easy to clean and reuse, in addition to requiring low energy and providing high flows, highlighting its potential as a sustainable emulsification technology for industrial applications (Wang et al., 2021a).

The production of $W_1/O/W_2$ emulsions through DMTS involves a three-step process: first, the bioactive material is incorporated into the internal aqueous phase of the emulsion (W_1/O), and then it is re-emulsified in a second aqueous phase (W_2), obtaining a $W_1/O/W_2$ coarse emulsion, both prepared using standard mixing techniques. Finally, the $W_1/O/W_2$ coarse emulsion is refined in the DMTS system, consisting of a bed of glass spheres supported by a metal sieve, which acts as a membrane. It is worth noting that the operating conditions, such as applied pressure, bed height, interstitial void diameter, and number of cycles, need to be adjusted to reduce the size of $W_1/O/W_2$ droplets and obtain a narrow droplet size distribution while retaining the encapsulated bioactive and providing a high throughput.

Due to the versatility of $W_1/O/W_2$ emulsions, the internal aqueous phase W_1 can carry water-soluble compounds, in this case, the polyphenols from beet by-product, while the oily phase (O) contains oil-soluble molecules (Kumar et al., 2022). Generally, canola, corn, soybean, sunflower, and mineral oils have been used to produce emulsions (Camelo-Silva et al., 2022). However, oils rich in omega-3 (polyunsaturated fatty acids - PUFAs), such as linseed oil, have become a trend due to their clinically proven health benefits (Kapoor et al., 2021). Combining polyphenol-rich extract and PUFAs in a single matrix, known as co-encapsulation, seems an interesting strategy that can maximize the beneficial effects on health and improve the nutritional profile of foods (Sultana et al., 2023). However, including PUFAs in $W_1/O/W_2$ emulsions is still challenging due to their high susceptibility to lipid oxidation. Natural antioxidants can minimize the effects of oxidation. They work by eliminating free radicals or controlling transition metals (Li et al., 2022). Therefore, we are interested in investigating whether the encapsulation of CEB rich in polyphenols with PUFAs will present synergism by reducing the effects of oxidation.

Stabilizing multiple emulsions, as opposed to single emulsions (W/O or O/W), is notably more challenging due to the need to stabilize two interfaces rather than just one (Eisinaite et al., 2016). Proteins are currently used in emulsions for food applications because even though they are non-synthetic, they reduce interfacial tension (O/W_2) while building up a viscoelastic film at the interface to enhance emulsion stability. Within the trend of alternative proteins from sustainable sources, recent studies have reported that insect proteins have emulsifying properties comparable/superior to whey protein isolate (WPI) (Ballon et al., 2024; Jayakumar et al., 2023; Wang et al., 2021a; Wang et al., 2021b), showing their potential as hydrophilic emulsifiers in double emulsions.

Increasing the antioxidant properties of proteins through conjugation with polyphenols has been suggested to produce emulsifiers capable of providing emulsions with improved physical and oxidative stability (Zhang et al., 2024). Protein-polyphenol conjugation can occur through covalent and non-covalent bonds. However, conjugates prepared by covalent bonding involve irreversible interaction forces and are more stable and suitable for the food industry (Liu et al., 2017). When protein-polyphenol conjugates are used as emulsifiers, emulsions undergo less lipid oxidation while physical stability is enhanced in some cases (Chen et al., 2023), while in others, it is not affected (Ballon et al., 2024). The possibility of increasing the antioxidant properties of insect proteins as emulsifiers was recently reported through the conjugation of lesser mealworm (*Alphitobius diaperinus*) protein concentrate (LMPC) with tannic acid (TA) or chlorogenic acid (CA). The antioxidant properties of the conjugates improved compared to the native proteins, reducing lipid oxidation without impairing the emulsifying activity (Ballon et al., 2024).

So far, no data are available on using insect protein-polyphenol conjugates to stabilize multiple emulsions, and this study is a pioneer in the area. Therefore, this work aimed to produce $W_1/O/W_2$ emulsions stabilized with LMPC and LMPC-TA for *co*-encapsulating polyphenols from the concentrated beet by-product extract (CEB) along with a PUFA-rich oil from linseed using a low-energy, high-throughput emulsification method. For achieving this objective, the effect of process conditions and interfacial composition on transmembrane flow, droplet size distribution, encapsulation efficiency, and physical and oxidative stability during 16 days of storage at 4 and 25 °C were investigated.

2. Material and methods

2.1. Materials

Aqueous extract of beet by-product (EB) was concentrated by forward osmosis and used as a source of polyphenols. Edible insect meal from lesser mealworm larvae (Kreca Ento-Food BV, Wageningen, The Netherlands) with a protein content of 45 % was used to produce LMPC. Linseed oil (Natursoy, Barcelona, Spain), obtained from a local supermarket, and polyglycerol polyricinoleate (PGPR, Palsgaard, Juelsminde, Denmark) were used to formulate the oil phase. Sodium hydroxide (NaOH), hydrochloric acid (37 %, HCl), citric acid, sodium phosphate dibasic (Na_2HPO_4), sodium carbonate (Na_2CO_3), and 2-methyl tetrahydrofuran (2-MeTHF) were purchased from Scharlab (Barcelona, Spain). 1,1,3,3-tetraethoxypropane (≥96 %, TEP), 2-thiobarbituric acid (≥98 %, TBA), 2,2-diphenyl-1-picrylhydrazyl (≥97 %, DPPH), isooctane, 2,2′-azobis(2-methylpropionamidine) dihydrochloride (97 %, AAPH), sodium azide, and tannic acid were obtained from Sigma-Aldrich (Saint Louis, MO, USA). Folin-Ciocalteau reagent and propan-2-ol were purchased from Chem-Lab NV (Zedelgem, Belgium). Trichloroacetic acid (≥99 %, TCA), Trolox (97 %), Pierce™ BCA protein assay kit, sodium phosphate monobasic (NaH_2PO_4), and dialysis bags (MWCO 3500 Da) were supplied by Thermo-Fisher Scientific (Waltham, MA, USA). All chemicals were of analytical grade or equivalent purity, and deionized water was used to prepare the samples and reagents.

2.2. Production of aqueous extract of beet by-product (EB) and forward osmosis (FO) concentration

EB was produced according to Camelo-Silva et al. (2024). The beets were obtained from a local supermarket in Florianópolis, SC, Brazil, and then cleaned with a chlorinated solution at 200 ppm for 15 min. Subsequently, the beets were rinsed with water to eliminate any remaining chlorine and air-dried at room temperature. Leaves and peels (approximately 1 mm thick) were dried in a forced-air circulation oven at 45 °C for 10 h, while stems were dried for 24 h. The by-products were then ground using a knife mill (Marconi, MA-580, Brazil) and stored at −18 ± 1 °C in polyethylene bags shielded from light with a layer of aluminum foil. The powdered material was used to extract the polyphenols through infusion in water (1:25 *w*/w) at 55 °C for 30 min. EB was stored in sterile Falcon tubes coated with a layer of aluminum and frozen at −18 ± 1 °C until concentration by FO.

EB concentration was carried out in an FO pilot unit (EvapEOs® - Micro pilot unit) provided by Ederna SAS (Toulouse, France), as illustrated by Wang et al. (2020). The FO unit was equipped with a 0.5 m^2 cellulose triacetate spiral membrane, reservoirs for the feed solution and draw solution (1 M calcium chloride), and pumps for both solutions. The pressure within the membrane module was carefully controlled below 70 kPa, and a water bath was connected to regulate the temperature of the feed solution, ensuring it remained below 20 °C. At the end of the process, we obtained a volume reduction (EB) factor of 5.

2.3. Characterization of EB and CEB

2.3.1. Total phenolic compounds (TPC)

The TPC content of EB and CEB was determined according to Camelo-Silva et al. (2024). Briefly, 250 μL of EB or CEB previously diluted in distilled water were homogenized with 250 μL of Folin-Ciocalteu reagent and 2 mL of distilled water and left to rest for 3 min at room temperature. Then, 250 μL of saturated sodium carbonate solution (20 g 100 mL^{-1}) was added, and the mixture was kept at 37 °C in a water bath for 30 min. Absorbance was measured on a spectrophotometer (Hach Lange DR5000, Hach Lange SLU, Spain) at 750 nm, and distilled water was used as a blank. The results were compared with a gallic acid standard curve and expressed as mg gallic acid equivalent (GAE) 100 mL^{-1} of extract.

2.3.2. Antioxidant capacity by DPPH and oxygen radical absorbance capacity (ORAC) methods

The antioxidant capacity by DPPH and ORAC methods was performed based on Ballon et al. (2024). DPPH radical capture was determined by mixing 750 μL of EB or CEB with 750 μL of DPPH solution (0.175 mM) prepared with methanol. The mixtures were then vortexed and stored in the dark for 1 h at room temperature, and the absorbance was measured at 517 nm. The ORAC assay was performed by mixing 25 μL of EB or CEB with 150 μL of fluorescein (68 nM) and leaving it to rest for 5 min. After incubation, 25 μL of AAPH (75 mM) was added, and fluorescence decay was measured at 520 nm with excitation at 485 nm on an FLUO star OPTIMA fluorescence reader (BMG Labtech, Offenburg, Germany). The area under the curve was calculated, and phosphate buffer was used as a blank. A Trolox solution was used as a standard curve, and the antioxidant capacity results (DPPH and ORAC) were expressed in μmol Trolox Equivalent Antioxidant Capacity (TEAC) 100 mL^{-1} of extract.

2.3.3. Total soluble solids content (°brix) and betalains

The °Brix of EB and CEB was determined by refractometry (WAY—1S, Zuzi, Auxilab S.L., Spain). The betalain content was obtained through the sum of betacyanin (*CBY*) and betaxanthin (*CBX*) (Arend et al., 2022). For this, McIlvaine buffer was gently added to 1 mL of EB or CEB until their absorbances reached the range of 0.8 to 1.0. Then, absorbance was measured at 535 nm and 484 nm for *CBY* and *CBX*, respectively. The concentration of *CBY* or *CBX* was determined according to Eq. 1.

$$CBY\ or\ CBX\ \left(g\,L^{-1}\right) = \frac{A*DF*MW}{\varepsilon*L} \quad (1)$$

where *CBY* is the betacyanin concentration (g L^{-1}), *CBX* is the betaxanthin concentration (g L^{-1}), *A* is the absorbance value, *DF* is the dilution factor, *MW* is the molecular weight (g mol^{-1}) being 550 g mol^{-1} for *CBY* and 308 g mol^{-1} for *CBX*, ε is the molar absorptivity being 60,000 L mol^{-1} cm^{-1} for *CBY* and 48,000 L mol^{-1} cm^{-1} for *CBX*, and L is the pathlength of the cuvette (cm).

2.4. Defatting and protein extraction of insect meal

The defatting and protein extraction was carried out following the method provided by Wang et al. (2021a) with some modifications. Briefly, the defatting was performed by mixing the edible meal from insect larvae (LM) powder with 2-MeTHF (ratio 1:5, *w/v*) and stirring at 600 rpm on a magnetic stirrer for 1 h. The mixture was then allowed to stand until complete phase separation, and the solvent layer containing lipids was carefully removed. This process was repeated three times to ensure the complete removal of lipids from LM. After the last repetition, the lipid-free LM was left in a fume hood overnight to remove residual solvent. For protein extraction, the lipid-free LM was mixed with 0.25 M NaOH (ratio 1:5, w/v) and stirred at 400 rpm on a magnetic stirrer at 40 °C for 1 h. Then, the mixture was centrifuged (3200 ×*g*, 15 min), and the supernatant was collected. The residual lipid-free LM pellet was subjected to this process twice more. The pH of the collected protein supernatants was adjusted to 4.0–4.5 with 37 % HCl followed by centrifugation (2233 ×*g*, 15 min). The LMPC precipitate was freeze-dried (LYOQUEST-85 PLUS, Telstar, Barcelona, Spain) for 24 h at 0.2 mbar, ground, and stored in a desiccator at 4 °C in plastic bags until further use.

2.5. Preparation of LMPC-TA

The LMPC-TA conjugate was obtained according to previous work (Ballon et al., 2024). LMPC was dispersed in distilled water (3.2 wt%) and stirred at 400 rpm for 2 h. The pH of the mixture was adjusted to 9.0 at 30-min intervals using 4 M NaOH, and then the mixture was refrigerated at 4 °C overnight. Then, the protein solution was centrifuged twice (2863 ×*g*, 15 min), and the protein content was quantified using the BCA method (Smith et al., 1985), with the protein content expressed as an equivalent value of bovine serum albumin (BSA). TA was mixed in distilled water, and the pH of the solution was adjusted to 9.0. Afterward, while the protein solution was stirred, the TA mixture was gently added until it reached a content of 150 μmol polyphenol/g protein. Distilled water was added to obtain a final content of 1 % protein and 0.02 % sodium azide to prevent microbial growth. The pH of the solution was adjusted to 9.0, and the solution was stirred at 400 rpm for 24 h at room temperature in the presence of oxygen. Subsequently, the pH of the solution was adjusted to 7.0, and unreacted polyphenols were removed through dialysis (MWCO 3500 Da) against distilled water for 44–48 h at 4 °C. Finally, LMPC-TA was freeze-dried and stored in a desiccator at 4 °C in plastic bags until further use.

2.6. Production of $W_1/O/W_2$ emulsions using DMTS

Eight emulsions were prepared (Table 1) following a protocol by Wang et al. (2021a) with some modifications. The LMPC-TA solution was prepared by dissolving 1 g of the sample in 99 g of distilled water,

Table 1
Formulation of $W_1/O/W_2$ emulsions and pressure applied during DMTS emulsification.

Formulation of emulsions				
Sample	Pressure (kPa)	Continuous phase (21 g)	W_1 (9 g)	W_2 (80 g)
Control-LMPC-450	450			LMPC solution (1.0 wt%) + 0.02 wt% sodium azide +1.40 wt% NaCl
Control-LMPC-300	300			
Control-LMPC-TA-450	450		distilled water (2.20 wt% NaCl)	LMPC-TA solution (1.0 wt%) + 0.02 wt % sodium azide +1.40 wt% NaCl
Control-LMPC-TA-300	300			
Beet-LMPC-450	450		CEB	LMPC solution (1.0 wt%) + 0.02 wt% sodium azide +1.40 wt% NaCl
Beet-LMPC-300	300			
Beet-LMPC-TA-450	450	14 wt% linseed oil with 4 wt% PGPR		LMPC- TA solution (1.0 wt%) + 0.02 wt % sodium azide +1.40 wt% NaCl
Beet-LMPC-TA-300	300			

stirring for 2 h, and then keeping it in the fridge overnight for complete hydration of the proteins. The LMPC solution was prepared by dissolving a desired amount of LMPC powder in distilled water, stirring for 2 h while adjusting the pH to 7.0 every 30 min, and then kept in the fridge overnight. The LMPC solution was centrifuged twice (2863 ×*g*, 15 min), and the protein content was quantified using a BCA assay kit. The required concentration of LMPC solution was obtained by dilution with distilled water. The high osmolality of CEB, resulting from the high concentration of sugar and polyphenols, was balanced by adding NaCl in W_2 (1.40 wt%) to avoid emulsion instability. The osmolality of the aqueous phases was measured using a vapor pressure osmometer (K-7000, KNAUER, Berlin, Germany) at 39 °C calibrated by a 400 mOsmol/kg NaCl solution.

The Fig. 1 shows the procedure for obtaining the refined emulsions. The coarse emulsion (W_1/O) was obtained by mixing W_1 (6 wt%) in linseed oil (14 wt%) with 4 wt% PGPR under homogenization in a rotor-stator (Ultra Turrax T18 digital, IKA, Staufen, Germany) at 11000 rpm for 5 min. Then, W_1/O was added to W_2 (80 wt%), containing 1.0 wt% LMPC or LMPC-TA and sodium azide (0.02 wt%), under magnetic stirring (1600 rpm for 5 min). The coarse emulsion was refined in a dynamic membrane of tunable pore size (DMTS). The emulsion ($W_1/O/W_2$) was forced to pass through the dynamic membrane constituted by a layer with 2 mm in height of silica microbeads (38 μm) supported by a nickel microsieve with rectangular pores of 284.7 × 12.8 μm (length x width). The interstitial void diameter of the porous system is about 22 μm. The system was pressurized to 300 or 450 kPa with nitrogen gas, and the first emulsification cycle was obtained. This process was repeated two more times (3 emulsification cycles). After each emulsification cycle, the mass of the refined emulsion was recorded over time on an electronic balance to monitor the mass flow rate. The transmembrane flux (J) was calculated according to Eq. 2.

$$J = \frac{\varphi}{\rho e^{*} A} \tag{2}$$

where, φ is the mass flow rate, acquired from the data recorded on the electronic balance, ρ_e the density of the emulsion, and A the effective permeation surface area.

Four control emulsions (without CEB) were produced following an identical process (Table 1), except that the W_1 phase consisted of a 2.20 wt% NaCl solution. The osmotic pressure of W_1 was adjusted to have a similar osmotic pressure to the CEB solution (732 mOsmol/kg). Once the emulsification process was completed (3 cycles), the module was disassembled, and the nickel sieve and microbeads were cleaned and reused. Droplet size distribution, morphology, and encapsulation efficiency (EE) characterized the freshly produced emulsions. Then, emulsions (from the last emulsification cycle, C3) were placed in capped tubes and stored for 16 days at 4 and 25 °C, respectively. Aliquots of the emulsions were analyzed every 4 days to measure their physical and oxidative stability. Emulsions were produced in duplicate.

2.7. Droplet size distribution

The diameter ($D_{4,3}$) and span of the $W_1/O/W_2$ emulsions were measured after each emulsification cycle by laser diffraction using Mastersizer 2000 (Malvern Instruments, Worcestershire, UK) as explained in Wang et al. (2021a). Destabilization of the emulsions during measurements was avoided by dispersing them in a 2.20 wt% NaCl aqueous solution as a continuous phase in the Mastersizer Hydro 2000 G accessory. The refractive index was defined as 1.480 and 1.330 for linseed oil and water, respectively.

2.8. Optical microscopy of $W_1/O/W_2$ emulsions

The microstructure of emulsions was observed using an inverted microscope (Primovert, Carl Zeiss AG, Oberkochen, Germany) under a 40× objective, equipped with a camera (SpeedCam MacroVis EoSens, High Speed Vision GmbH, Ettlingen, Germany).

2.9. Encapsulation efficiency (EE) of polyphenols

EE was obtained according to Berendsen et al. (2015). The emulsions were centrifuged for 10 min at 825 ×*g*. Afterward, the aqueous phase (W_2) was collected and analyzed for TPC (section 2.3.1). The mass of polyphenols that remained encapsulated in W_1 was expressed as the EE of polyphenols using Eq. 3.

$$EE\ (\%) = \frac{m^{0}_{poly_{W_1}} - C^{n}_{poly_{W_2}}\left(m^{0}_{W_1} + m^{0}_{W_2}\right)}{m^{0}_{poly_{W_1}} - C^{n}_{poly_{W_2}} * m^{0}_{W_1}} \tag{3}$$

where $m^{0}_{poly_{W_1}}$ is the initial polyphenol mass in the inner water phase (W_1), $C^{n}_{poly_{W_2}}$ is the concentration of polyphenols in the outer water phase (W_2), $m^{0}_{W_1}$ is the initial mass of the inner water phase, and $m^{0}_{W_2}$ is the mass of the outer water phase after n cycles.

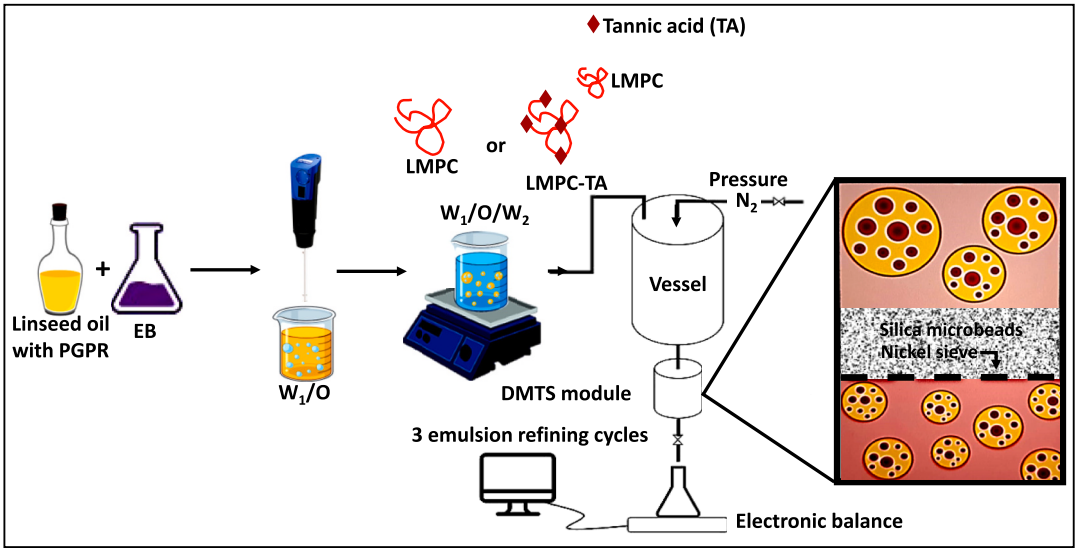


Fig. 1. Process flowchart for obtaining $W_1/O/W_2$ emulsions with DMTS.

2.10. ζ-potential determination

The ζ-potential of emulsions was measured using dynamic light scattering (Zetasizer Nano-ZS, Malvern Instruments, Worcestershire, UK) at 25 °C (Ballon et al., 2024).

2.11. Physical and oxidative stability of emulsions during storage

2.11.1. Droplet size distribution

The physical stability (droplet size and span) of the $W_1/O/W_2$ emulsions was determined according to section 2.7.

2.11.2. Concentration of polyphenols in W_2

The polyphenols content in W_2 was obtained by centrifuging the emulsions (825 ×*g* for 10 min), followed by TPC quantification in the aqueous phase according to the methodology described in section 2.3.1.

2.11.3. Primary oxidation products by conjugated dienes hydroperoxides

Conjugated dienes hydroperoxides were determined following a methodology adapted by Ballon et al. (2024). 200 μL of the emulsion was added to 1 mL of isooctane/2-propanol (3:1 *v*/v), vortexed for 1 min, and then centrifuged (1000 x*g*, 5 min). The supernatant was collected and diluted with isooctane, and absorbance was measured at 234 nm. The conjugated dienes hydroperoxides concentration was calculated by Beer-Lambert law using 25,200 M^{-1} cm^{-1} as the molar extinction coefficient of lipid hydroperoxides and was expressed in mmol hydroperoxide (HP) per kg of oil.

2.11.4. Secondary oxidation products by 2-thiobarbituric acid reactive substances (TBARS)

Secondary oxidation compounds were determined according to Ballon et al. (2024). 250 μL of emulsion was mixed with 250 μL of distilled water, and then 1 mL of TBA reagent (0.375 wt% TBA, 15 wt% TCA in 0.25 M HCl) was added. The mixture was centrifuged (1000 ×*g*, 5 min), and the aqueous phase was collected, placed in capped tubes, and soaked in a water bath (100 °C for 15 min). Then, the samples were cooled in an ice bath for 5 min, centrifuged (6250 x*g*, 5 min), and the absorbance was measured at 532 nm. Results were compared with a standard curve of 1,1,3,3-TEP and expressed as the ratio of TBARS on the day of analysis to the initial TBARS value.

2.12. Statistical analysis

The results were expressed as mean ± standard deviation from duplicate emulsions analyzed in triplicate. One-way analysis of variance (ANOVA) was performed using STATISTICA® software version 13.3 (TIBCO Software Inc., USA). Differences between means were determined using Tukey's test at a significance level of 0.05.

3. Results and discussion

3.1. Characterization of CEB

This section shows the levels of bioactive compounds of CEB, which was used as a source of polyphenols for producing $W_1/O/W_2$ emulsions (Table 2). The main objective here was to characterize EB after concentration by FO, serving as a database for this innovative technology of food concentration. As shown in Table 2, following the concentration process by FO, an approximately fivefold increase was observed in polyphenol content, both in total (from 43.1 to 221.0 mg GAE 100 mL^{-1}) and specifically in betalain (from 340.0 to 1790.0 mg 100 mL^{-1}). During FO, water is transferred through a semipermeable membrane from the feed solution (EB) to the draw solution (calcium chloride), driven by the difference in osmotic pressure. The removal of water from EB causes an increase in the concentration of total solids, which are represented mainly by TPC, betalains, and sugars (°Brix). An increase in the content of TPC and betalains is directly related to the antioxidant activity of the final concentrate, CEB. CEB presented antioxidant activity values of 50.50 and 5.93 μmol TEAC 100 mL^{-1} of extract, using the DPPH and ORAC methods, respectively, and a soluble solids content of 6°Brix.

Table 2
Polyphenol contents, antioxidant activity, betalain, and soluble solids (°Brix) of EB concentrated (CEB) by forward osmosis.

TPC (mg GAE 100 mL^{-1} of extract)	221.00 ± 4.32[a]
DPPH (μmol TEAC 100 mL^{-1} of extract)	50.50 ± 6.42[a]
ORAC (μmol TEAC 100 mL^{-1} of extract)	5.93 ± 0.28[a]
Betalain (mg 100 mL^{-1} of extract)	1790.00 ± 60.80[a]
Soluble solids (°Brix)	6.60 ± 0.30[a]

Results are expressed as mean ± standard deviation. TPC: total phenolic compounds. DPPH: 2,2-diphenyl-1-picrylhydrazyl. ORAC: oxygen radical absorbance capacity.

The concentrated beet extract has a higher polyphenol content that could benefit from encapsulation in $W_1/O/W_2$ emulsions, as health improvements in several aspects have often been related to phenolic compounds from plant extracts. Besides, using beet by-product extract rich in antioxidant compounds can improve the oxidative stability of the emulsion by eliminating free radicals.

3.2. Characterization of $W_1/O/W_2$ emulsions

3.2.1. Droplet size distribution and morphology

The freshly produced $W_1/O/W_2$ emulsions were characterized by their droplet size distribution and morphology. In Fig. 2 we show microscopic images of emulsions produced at 450 kPa after 3 cycles of refinement. For all tested conditions, it can be seen the typical microstructure of double emulsions where larger oil droplets are filled by smaller water droplets.

Fig. 3 shows that regardless of the sample, diameter ($D_{4,3}$) and span showed a typical pattern of reduction as the refinement cycles increased, in which the greatest reduction in droplet size was observed after the first cycle, followed by a reduction constant, but smaller, during cycles 2 and 3. These results demonstrated that the DMTS system is robust and effectively refining double emulsions. $D_{4,3}$ and the span of the coarse emulsions, obtained through agitation (1600 rpm for 5 min), ranged from 30 to 50 μm and 1.6 to 2.4, respectively. $D_{4,3}$ of all coarse emulsions was reduced at least 3.5 times after the first emulsification cycle, presenting a $D_{4,3}$ below 8.35 μm after the third cycle. Our results revealed that $D_{4,3}$ of the emulsions during the emulsification cycles was not affected ($p > 0.05$) by pressure or type of protein (LMPC or LMPC-TA), suggesting that the use of 300 kPa in the refinement of emulsions $W_1/O/W_2$ is enough to assure a similar extent in droplet size reduction to that obtained at higher pressures, in turn reducing energy costs. These results are in good agreement with a previous work (Ballon et al., 2024) in which O/W emulsions stabilized with LMPC showed a similar pattern of droplet size reduction during emulsification with DTMS to those stabilized with LMPC-TA conjugate, even though the conjugation process modified some physicochemical properties (e.g., surface hydrophobicity, interfacial tension) of LMPC compared with LMPC-TA conjugate. As for the number of emulsification cycles, non-significant differences in $D_{4,3}$ were found between cycles 2 and 3, indicating that the refinement process could be stopped after the second cycle, with the consequent reduction in energy input. Regarding droplet size dispersion, all emulsions presented span values ≤1 after the first emulsification cycle, that is, a droplet distribution close to monomodal. It is worth emphasizing that $W_1/O/W_2$ emulsions stabilized with LMPC-TA exhibited significantly lower span values (0.83–1.84) than those produced with LMPC (0.96–2.34) for the whole range of emulsification cycles (from coarse to cycle 3). The presence of LMPC and LMPC-TA at the O/W_2 interface affects the surface charge of the droplets, as the values of ζ-potential indicate. Emulsion Beet-LMPC-TA-450 showed an

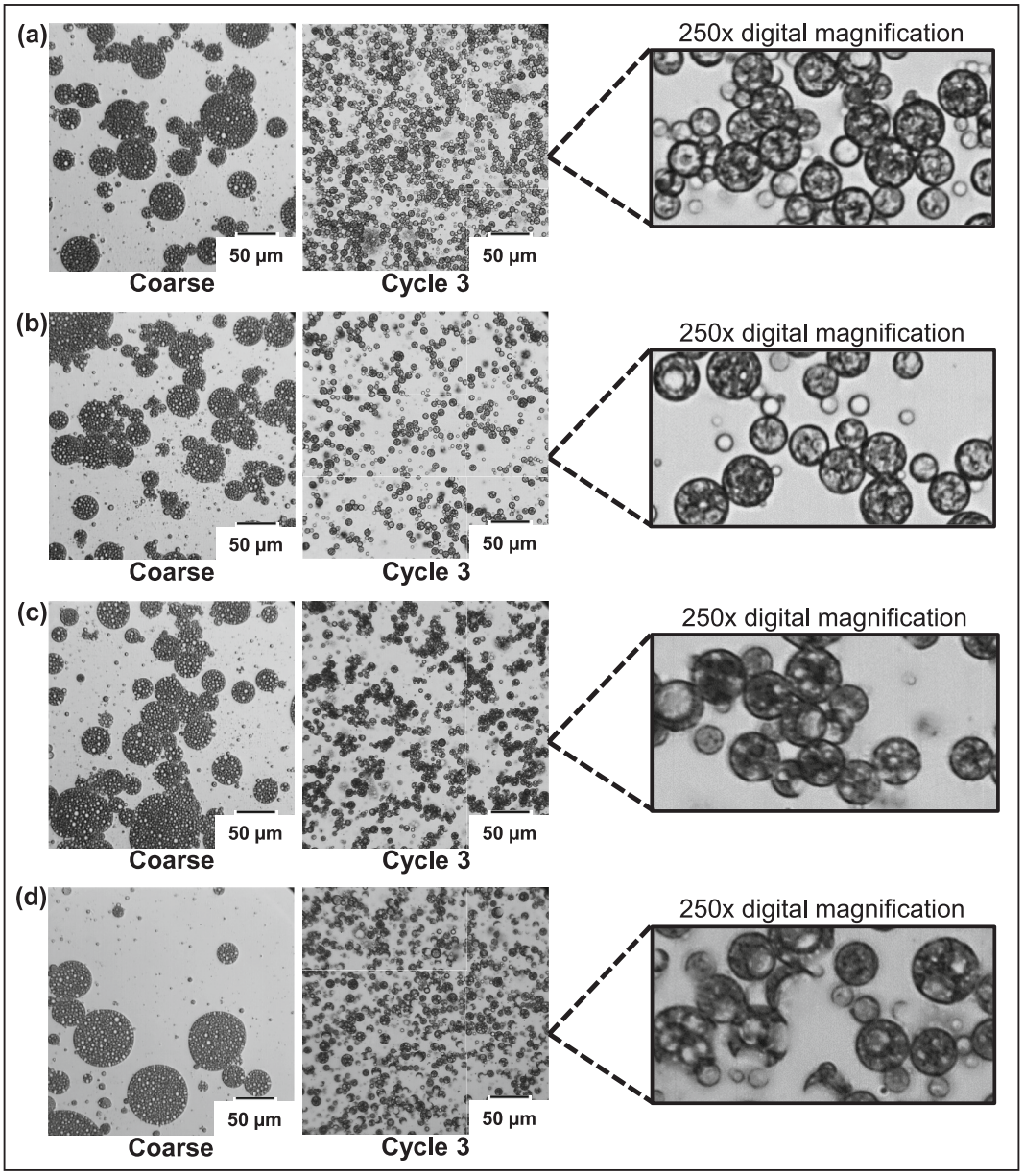


Fig. 2. Optical microscopy of $W_1/O/W_2$ emulsions stabilized with Control-LMPC (a), Control-LMPC-TA (b), Beet-LMPC (c), and Beet-LMPC-TA (d).

absolute value of ζ-potential higher (−54.3 mV) than the Beet-LMPC-450 (−30 mV). The greater negative charge of LMPC-TA may have strengthened the electrostatic repulsion between emulsion droplets, minimizing their aggregation and producing more stable double emulsions (Ballon et al., 2024; Wang et al., 2022). The droplet size distribution behavior found here corroborates that of Wang et al. (2021a), who produced double emulsions ($W_1/O/W_2$) loaded with a procyanidin-rich extract refined in a similar DMTS system.

3.2.2. *Transmembrane flux (TF) of $W_1/O/W_2$ emulsions*

TF is an extremely important process parameter that can be used as a productivity indicator. The values obtained using the DMTS system (129 to 372 $m^3\ h^{-1}\ m^{-2}$) are high (of industrial interest) and comparable to others obtained using a similar set-up. The effect of the pressure applied to the system on the TF during the refinement of $W_1/O/W_2$ emulsions is shown in Fig. 3. Although differences in TF between 300 and 450 kPa were not great, they were significant, especially at cycle 2. Regarding the progress during DMTS emulsification, TF showed a significant increase from cycle 1 to 2, while TF remained unchanged from cycle 2 ($p > 0.05$). For the $W_1/O/W_2$ emulsion to flow through the interstices formed (~ 22 µm) by the silica bed, energy is required to break up the emulsion droplets and flow through the DMTS system. From the pattern in droplet reduction, it can be seen how, in cycle 1, most of the energy is invested in droplet break up with the corresponding lowest values of TF. As soon as the droplet size does not reduce, cycles 2 and 3, TF stays unchanged. It is worth emphasizing that regardless of the cycles, the TF found in this paper are greater than 100 $m^3\ h^{-1}\ m^{-2}$, that is, in the range of industrial interest, and similar to those reported for emulsification in DMTS to encapsulate a commercial extract rich in procyanidin (95 to 402 $m^3\ h^{-1}\ m^{-2}$) (Wang et al., 2021a). On the other hand, they were higher than those found for emulsification in DMTS for a mixture of whey protein isolate (WPI) with beet juice (0.5 to 65 $m^3\ h^{-1}\ m^{-2}$) (Eisinaite et al., 2016). TF results are not always comparable with those of other studies, as they depend on factors such as emulsion composition, DMTS configuration, and pressure applied to the system (Wang et al., 2021a).

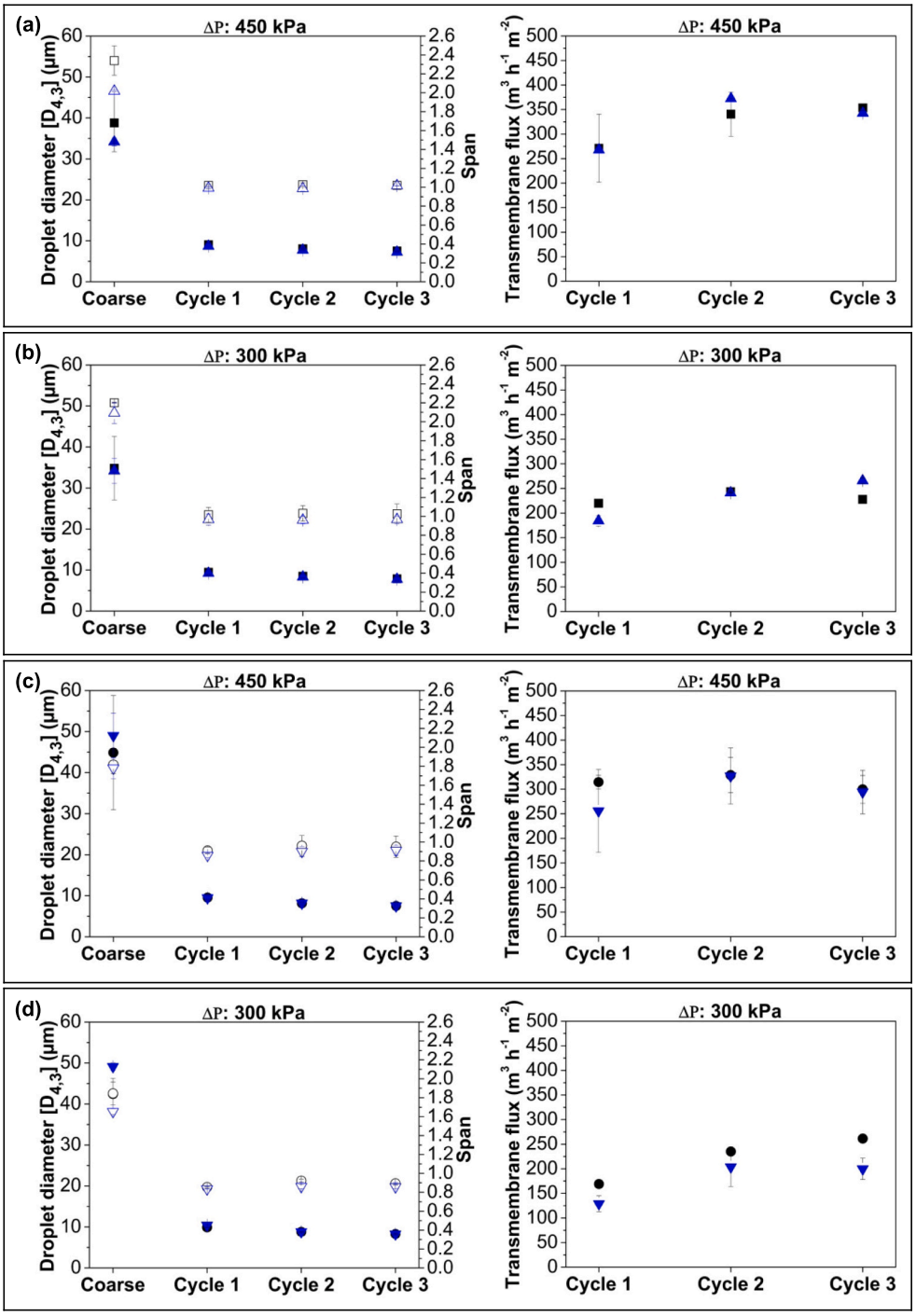
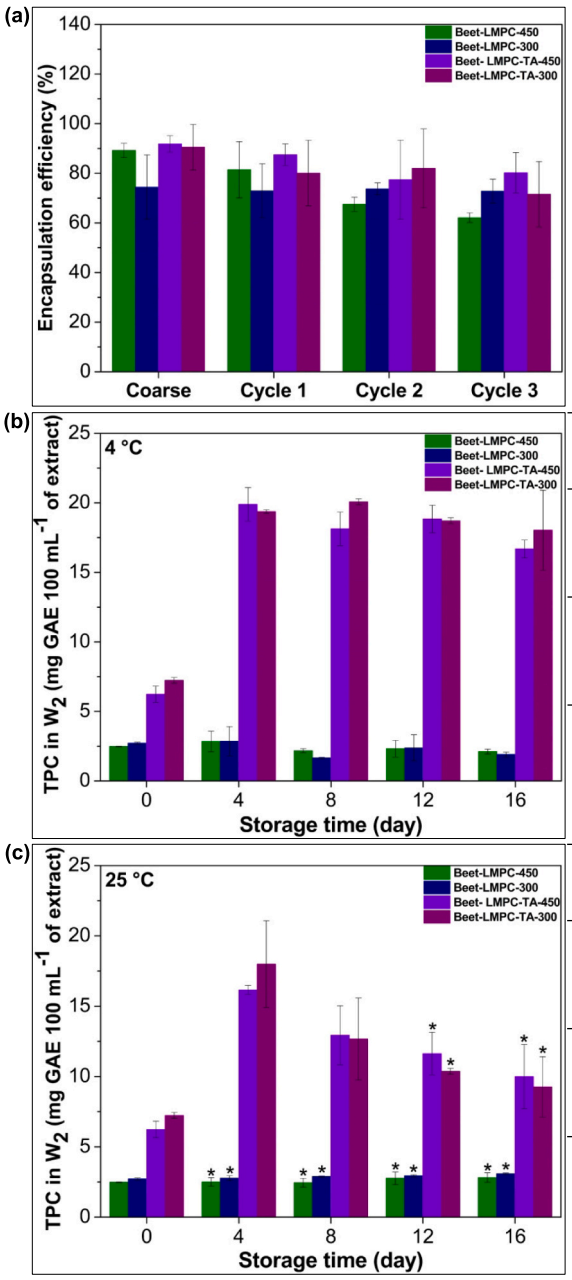


Fig. 3. Droplet size distribution and transmembrane flux of $W_1/O/W_2$ emulsions: (a) LMPC-stabilized at 450 kPa, (b) LMPC-stabilized at 300 kPa (c) LMPC-TA-stabilized at 450 kPa and (d) LMPC-TA-stabilized at 300 kPa. (■) Control-LMPC, (▲) Beet- LMPC, (●) Control-LMPC-TA, and (▼) Beet-LMPC-TA. Filled markers refer to droplet size, and empty markers refer to droplet span.

3.2.3. *Encapsulation efficiency (EE) of polyphenols*

The encapsulation efficiency of CEB was calculated as the mass of polyphenols that remained encapsulated within the W_1 droplets over the total mass of polyphenols initially introduced in the inner water phase. Fig. 4 (a) shows the EE of polyphenols in the coarse $W_1/O/W_2$ emulsions and after the three emulsification cycles. Similarly to what was observed in the evolution of the droplet size distribution, the ANOVA statistical analysis confirmed that the type of protein used to stabilize the O/W_2 interface (LMPC or LMPC-TA) and the applied pressure did not have a significant impact on the EE values ($p > 0.05$). On the other hand, a slight reduction in EE was observed during the emulsification cycles. For example, higher EE was obtained for the coarse emulsion and emulsification cycles 1 and 2, while lower polyphenol content remained within W_1 after the third cycle, except for the Beet-LMPC-TA-450 sample. This decrease in EE was believed to have resulted from the release of polyphenols from W_1 caused by droplet rupture during emulsification. During the refinement of $W_1/O/W_2$ emulsions, a few drops of W_1 containing CEB may be expelled into the continuous phase, discharging the polyphenols into W_2. This would result in a higher reduction in EE as droplet break up increases, as can be seen from the progress of EE and



Day	Sample	TPC in W_2 (mg GAE 100 mL^{-1} of extract)
0	Control LMPC	2.04 ± 0.01
	Control LMPC-TA	6.82 ± 0.02
16	Control LMPC	1.75 ± 0.01
	Control LMPC-TA	6.03 ± 0.40

Day	Sample	TPC in W_2 (mg GAE 100 mL^{-1} of extract)
0	Control LMPC	2.04 ± 0.01
	Control LMPC-TA	6.82 ± 0.02
16	Control LMPC	2.18 ± 0.14
	Control LMPC-TA	5.40 ± 0.50

Fig. 4. Encapsulation efficiency (a) and TPC concentration in W_2 during storage for 16 days at 4 °C (b) and 25 °C (c). TPC at times 0 and 16 days for control samples (without CEB) are included on the right side of Fig. (b) and (c). Asterisk labels above the bars indicate a precipitate's appearance during polyphenol analysis.

$D_{4,3}$ during emulsification (Fig. 3). In line with this, two cycles of emulsification enable a significant reduction in droplet size (with no significant differences in $D_{4,3}$ between cycles 2 and 3 and values of span below 1) while keeping EE between 67.5 % and 82 % regardless of the interfacial composition. These results agree with previous findings for encapsulating phenolic-rich grape seed extract in refined ($W_1/O/W_2$) emulsions on a hydrophilic porous Shiratsu glass (SPG) membrane (Estévez et al., 2019).

3.3. Physical and oxidative stability of emulsions during storage

This section presents the physical and oxidative stability results of $W_1/O/W_2$ emulsions. Emulsion samples from the last emulsification cycle (cycle 3) were stored for 16 days at 4 and 25 °C and evaluated regarding droplet size distribution (section 2.7), polyphenol concentration in W_2 (section 2.11.2), and production of primary (section 2.11.3) and secondary (section 2.11.4) lipid oxidation products.

3.3.1. Droplet size distribution

Fig. 5 shows the stability of the $W_1/O/W_2$ emulsions from their droplet size distribution during storage at 4 and 25 °C for 16 days. The data ($D_{4,3}$ and span) were plotted in separate figures according to the storage temperature (4 or 25 °C) and pressure used in refinement (300 or 450 kPa).

Initially, all emulsions had similar droplet sizes (7.24–8.35 μm) and had a monomodal distribution (0.85–1.0); however, the $D_{4,3}$ of emulsions refined at 450 kPa was slightly lower ($p < 0.05$). It was observed that the storage temperature strongly affected the physical stability of the emulsions in terms of $D_{4,3}$ and span. The emulsions stored at 4 °C (Fig. 5 (a) and (c)) showed high physical stability throughout the storage period (16 days), and it is clear that for the current scenario, the two proteins assessed (LMPC or LMPC-TA) are effective in stabilizing the

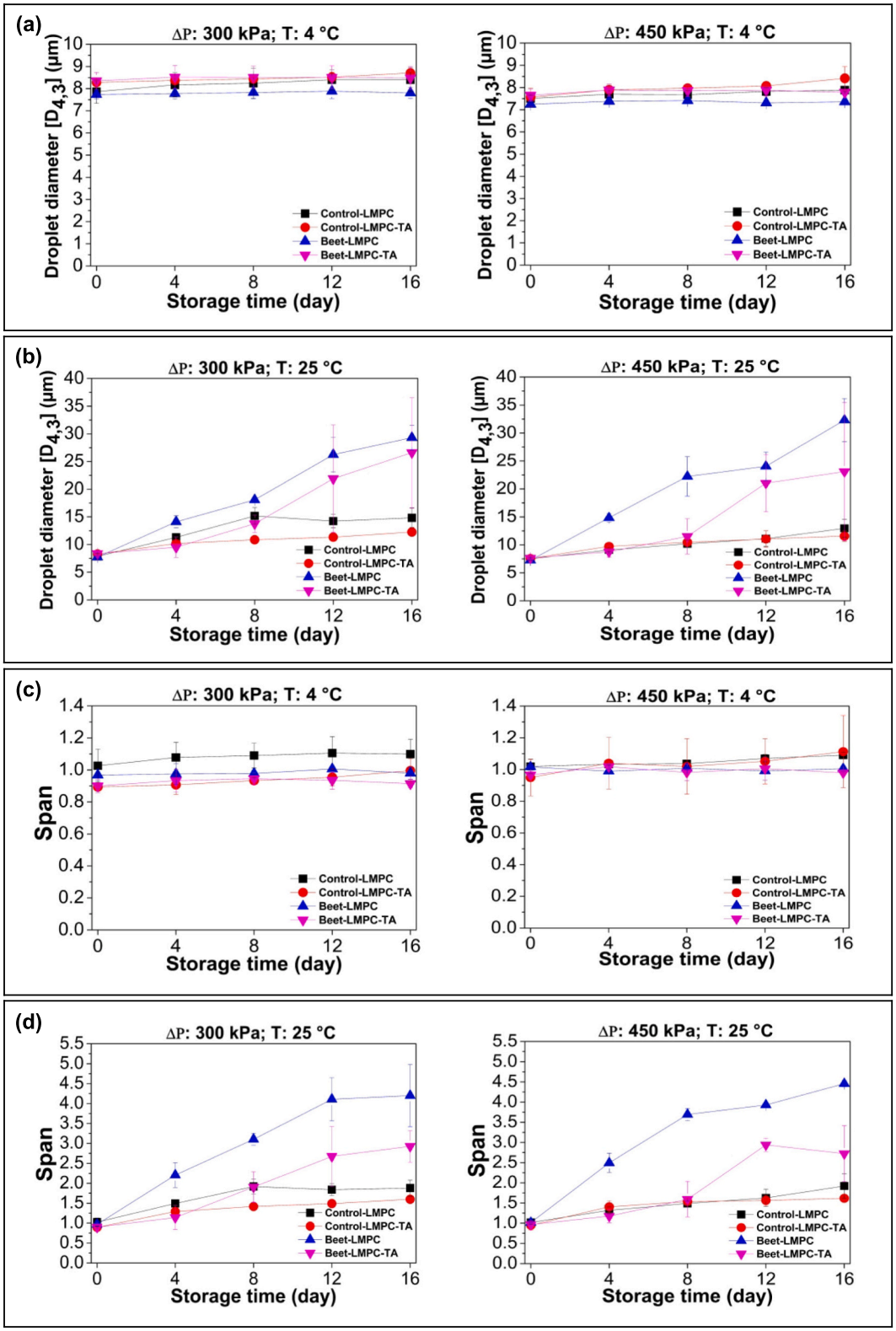


Fig. 5. Droplet size distribution of $W_1/O/W_2$ emulsions during storage for16 days at 4 and 25 °C: $D_{4,3}$ at 4 °C (a) and 25 °C (b), and span at 4 °C (c) and 25 °C (d). Lines are used for better visualization.

interface O/W_2 of emulsions at refrigeration temperature. In turn, at 25 °C (Fig. 5 (b) and (d)), regardless of the pressure applied during emulsification, the $W_1/O/W_2$ emulsions showed a similar trend of increasing $D_{4,3}$ and span with the increasing storage time. In all cases, the emulsions containing CEB showed a higher increase in $D_{4,3}$ and span at 25 °C; however, those emulsions stabilized with LMPC-TA showed a statistically lower increase than the emulsions stabilized with LMPC. Although the mechanisms of emulsion instability (coalescence of the outer droplets, coalescence of the inner droplets, coalescence of inner droplets with the outer droplet interface, shrinkage or swelling of inner

droplets) could not be identified from the monitored data, the progress of the outer droplet size distribution enables to identify some effects. First, entrapping CEB in W_1 resulted in a faster destabilization process than in control emulsions, even though formulation was adjusted to prevent osmotic unbalance between W_1 and W_2 (see Table 1). After sixteen days of storage, control emulsions showed about a two-fold increase of $D_{4,3}$ and span (Fig. 5 (b) and (d)), while in the case of emulsions with CEB, $D_{4,3}$ was up to 6 times higher than initially. These results align with the concentration of polyphenols in W_2 (discussed in section 3.3.2) since the appearance of precipitate due to the protein-polyphenol interaction may have contributed to the increase in $D_{4,3}$ and span. Besides, we also observed differences between LMPC and LMPC-TA in their capacity to stabilize the O/W_2 interface. After sixteen days of storage at 25 °C, the $D_{4,3}$ emulsions stabilized with LMPC-TA were slightly lower (11.57–26.60 μm) than those stabilized with LMPC (12.96–32.30 μm). The same trend was observed for the span, which ranged from 1.59 to 2.92 for emulsions produced with LMPC-TA and from 1.88 to 4.45 for emulsions containing LMPC. A previous study reported that $W_1/O/W_2$ emulsions produced with betanin, soybean oil, and emulsifiers CR-310 and Tween 20 were physically stable for up to seven days under refrigeration and unstable at 25 and 60 °C for the same storage period (Pagano et al., 2018). The results of the present study seem to indicate that LMPC-TA stabilized emulsions are the most promising in ensuring physical stability during storage at 25 °C.

3.3.2. Concentration of polyphenols in W_2

Fig. 4 (b) and (c) show the concentration of TPC in W_2 during storage for 16 days at 4 and 25 °C, respectively. The higher concentration of initial TPC in W_2 for samples Beet-LMPC-TA-300 and Beet-LMPC-TA-450 is due to the contribution of tannic acid (TA) presence, which was confirmed when control samples without CEB (Control- LMPC-TA) were tested. From Fig. 4 (b), it was found that after four days of storage at 4 °C, the TPC concentration increased rapidly in W_2 for the Beet-LMPC-TA-300 and Beet-LMPC-TA-450 emulsions. However, these values remained constant until the end of storage. The higher TPC concentration in W_2 for the Beet-LMPC-TA-300 and Beet-LMPC-TA-450 emulsions may be due to the polyphenol concentration gradient between W_1 and W_2, i.e., part of the CEB polyphenols were quickly transferred to W_2 to then balance with W_1. This hypothesis was later confirmed by finding that the TPC values in W_2 for the Control-LMPC-TA sample, without CEB, did not change between the first and last day of storage (values included in Fig. 4 (b) and (c)). Therefore, our results suggest that the release of polyphenols from CEB to W_2 occurred during the first four days and that the constant maintenance of TPC values in W_2 throughout storage (Fig. 4 (b)) is due to the good thermal stability of CEB polyphenols at mild temperatures (4 °C). On the other hand, at 25 °C, Beet-LMPC-TA-300 and Beet-LMPC-TA-450 emulsions showed a decline in TPC of W_2 after 8 days of storage (Fig. 4 (c)). It is believed that those polyphenols released in the first four days (represented mainly by betalains) were degraded throughout storage since this decrease in the TPC of W_2 cannot be attributed to the degradation of TA present in the LMPC-TA conjugate. The proof of the latter is that TPC in W_2 of the control sample (Control-LMPC-TA) did not significantly change during 16 days of storage at 25 °C. Moreover, a recent study revealed that betalain content decreased by approximately 98.8 % and 90.8 % after fifteen days of storage at 40 °C and room temperature, respectively (Mohammed et al., 2021). Furthermore, with increasing storage temperature, they observed that the red color of the beet extract was reduced and changed to a lighter yellow color. They attributed the color change to the degradation of betalain at higher temperatures to form neobetanin, a yellow degradation product. These findings agree with our results since our freshly produced emulsions were pinkish, and at the end of storage at 25 °C, they showed a color trend toward light yellow (data not shown).

For the LMPC-stabilized samples (Beet-LMPC-300, Beet-LMPC-450, and Control-LMPC) stored under the same conditions (4 and 25 °C), no significant variations were noted in the TPC values in W_2 during storage. However, at 25 °C, a precipitate was observed after 4 days of storage, while for emulsions stabilized with LMPC-TA, this phenomenon was verified after twelve days of storage (Fig. 4 (c)). To explain these results, we should consider that proteins can interact with polyphenols, in this case, with those in CEB, and form aggregates through non-covalent interactions, such as hydrogen bonds, hydrophobic bonds, and van der Waals forces, as well as through covalent bonds. The intensity of those interactions can be affected by temperature, pH, salt concentration, and the presence of certain reagents (Ozdal et al., 2013; Wang et al., 2021a), which, in turn, determines if the resulting protein-polyphenol complexes (non-covalent bonding) and conjugates (covalent bonding) are soluble or form precipitates. In the present case, we identify two scenarios regarding protein-polyphenol interactions. On one side, we have the $W_1/O/W_2$ emulsion containing CEB in W_1 and LMPC at the interface and in W_2, where LMPC was available to interact with the CEB polyphenols released in W_2 during and after emulsification. On the other side, at the interface and in W_2, we found LMPC conjugated with TA, which presents less available sites to bind the CEB polyphenol released to W_2 during and after emulsification. According to this, the higher rate of precipitate formation observed in LMPC-stabilized emulsions at 25 °C can be explained by the greater ability of LMPC to interact with polyphenols (compared to the conjugate LMPC-TA) together with a higher concentration of available CEB polyphenols in W_2. This can be correlated with the results on physical stability (section 3.1.1), which show an increase in $D_{4,3}$ and span, that is, certain emulsion instability resulting in polyphenol release and, simultaneously, in precipitate formation. At this point, it is important to mention that the pH of the emulsions was in the range of 6.5 to 7.5 and that the osmolarity of the W_1 and W_2 phases was balanced by adding NaCl. Based on these results, we concluded that the determination of the real TPC content in W_2 for samples incubated at 25 °C was compromised by the formation of protein-polyphenol precipitates since only polyphenols in solution were quantified. Similar results were reported for double emulsions loaded with a procyanidin-rich extract stabilized with LMPC, WPI, and pea protein (Wang et al., 2021a).

Even though using LMPC-polyphenol conjugates as emulsion stabilizers was not able to prevent the formation of precipitates during storage at 25 °C, it significantly delayed when they appeared. In this regard, the next step will be to develop LMPC-polyphenol conjugates with the same polyphenols to be encapsulated to minimize the interactions between the protein and the released polyphenols during storage. When stored under refrigeration (4 °C), LMPC-TA conjugate did not improve the capacity of the native LMPC to retain the entrapped BCE polyphenols.

3.3.3. Oxidative stability

Linseed oil is highly sensitive to oxidation due to its polyunsaturated nature, which can result in the loss of its nutritional value and the development of unpleasant odors (Sultana et al., 2023). Fig. 6 shows the formation of conjugated dienes (Fig. 6 (a) and (b)) and TBARS (Fig. 6 (c) and (d)) during storage for 16 days at 4 and 25 °C for the double emulsions produced in the present study. Conjugated dienes are compounds formed by rearranging of hydroperoxide double bonds during oil oxidation (Safarpour et al., 2022). In general, regardless of the storage temperature and emulsion composition, there were no substantial variations in conjugate diene formation throughout storage, remaining in the range of 12.27 to 18.15 mmol hydroperoxide/kg oil, with slightly higher values for the Control-LMPC-300 sample. Similar behavior was observed for linseed oil emulsions (O/W) stabilized with LMPC-tannic acid and LMPC-chlorogenic acid conjugates, with conjugated dienes values varying between 13 and 19 mmol hydroperoxide/kg oil (Ballon et al., 2024). These authors concluded that as hydroperoxides are primary compounds of lipid oxidation, the small variations in conjugated dienes may result from the process's kinetics when their formation rate becomes slower than their decomposition rate.

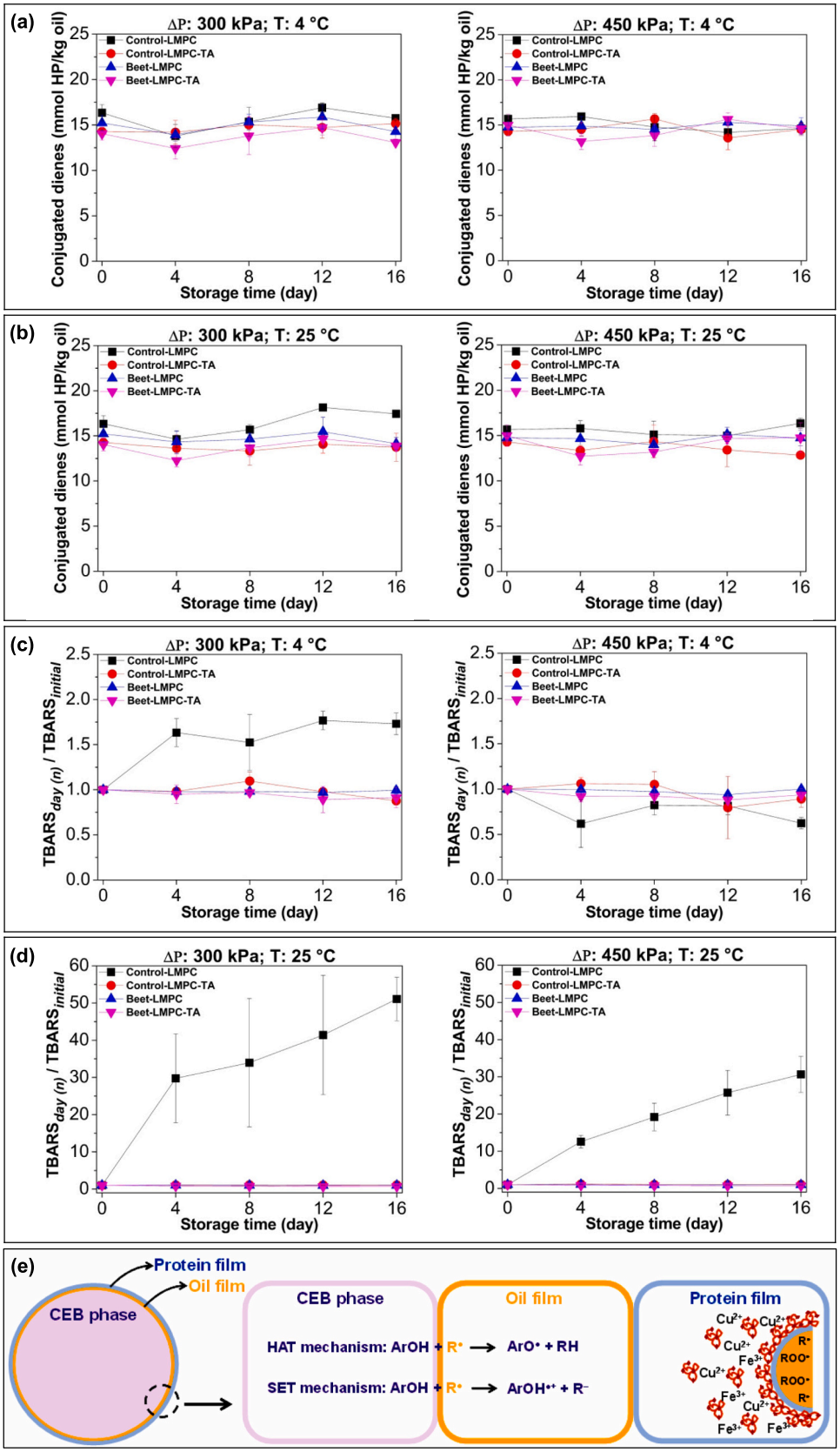


Fig. 6. Oxidative stability of $W_1/O/W_2$ emulsions during storage for 16 days at 4 and 25 °C. Conjugated dienes at 4 °C (a) and 25 °C (b). TBARS at 4 °C (c) and 25 °C (d). (e) Mechanisms controlling oxidation in emulsions loaded with concentrated beet extract (CEB) stabilized with protein-polyphenol conjugates (LMPC-TA). HAT, transfer of a hydrogen atom; SET, transfer of a single electron; ArOH, phenolic compound; $R^{\bullet}$, free radical; $ROO^{\bullet}$, peroxide radical; Fe^{3+} and Cu^{2+}, pro-oxidant metals. Lines are used for better visualization.

The TBARS values measure the development of oil oxidation in terms of secondary oxidation products, and they are defined as the quantity of malondialdehyde (in mg) present in 1 kg of sample (Zhang et al., 2010). The TBARS levels of the $W_1/O/W_2$ emulsions were strongly affected by the incubation temperature and composition of the emulsions (Fig. 6 (c) and (d)). When emulsions were stored at 4 °C, comparable TBARS levels were seen except for the Control-LMPC-300 sample. These results corroborate conjugated dienes since this sample presented slightly higher levels of lipid hydroperoxides. Significantly lower TBARS levels were observed when this sample was refined at 450 kPa and stored under the same conditions. This same trend was observed for the Control-LMPC-300 and Control-LMPC-450 samples during storage at 25 °C, which showed high oxidation levels. After forming primary hydroperoxides, they can migrate to the O—W_2 interface, interact with metals in the aqueous phase, and decompose into alkoxyl or hydroxyl radicals. These radicals are involved in the oxidation of fatty acids, producing lipid oxidation secondary products such as malondialdehyde, which is detected by the TBARS assay (Gutiérrez-del-Río et al., 2021).

At 25 °C, the emulsions containing CEB or LMPC-TA showed excellent oxidative stability throughout storage, suggesting that the CEB polyphenols and the LMPC-TA conjugate were effective in reducing the formation of alkoxyl radicals and hydroxyl, minimizing subsequent reactions. According to Gutiérrez-del-Río et al. (2021), the oxidative stability conferred by phenolic compounds can be attributed to two main mechanisms: direct reaction with free radicals, which act as primary antioxidants, or chelating free metals, which function as secondary antioxidants (Fig. 6 (e)). In the first case, polyphenols inactivate free radicals by mechanisms that involve the transfer of a hydrogen atom (HAT) or a single electron (SET) to the free radical ($R^{\bullet}$), stabilizing it. In the HAT mechanism, the phenolic compound (ArOH) donates a hydrogen atom to the free radical ($R^{\bullet}$), resulting in the formation of the oxidized radical ($ArO^{\bullet}$) and the inactive species (RH), which are much more stable than $R^{\bullet}$. Alternatively, in the SET mechanism, an electron is transferred to the radical ($R^{\bullet}$), creating a stable R^{-} anion and a radical cation ($ArOH^{\bullet+}$), which deprotonates upon interacting with water. Both $ArO^{\bullet}$ and $ArOH^{\bullet+}$ are aromatic structures that allow the free radical to move throughout the molecule, stabilizing it.

On the other hand, according to Lin et al. (2023), the protein-polyphenol conjugate can improve the oxidative stability of emulsions through three mechanisms (Fig. 6 (e)). First, the protein conjugated to a polyphenol is more efficient in stabilizing emulsions than its corresponding native form. LMPC-TA may have created an interfacial film (O/W_2) around the linseed oil droplets, separating lipids from pro-oxidants (e.g., Fe^{3+} and Cu^{2+}) in the aqueous phase (Ballon et al., 2024). Second, the protein-polyphenol conjugate can serve as a chemical barrier on the droplet surfaces, slowing the transition of metals and free radicals. Polyphenols, containing one or more hydroxyl groups, can donate their hydrogen atoms or transfer electrons, reducing metals and/or neutralizing free radicals. Third, the change in protein structural rearrangement may modify the conformation of peptides on droplet surfaces, exposing the nucleophilic centers of peptides (particularly long-chain ones), which may play an antioxidant role.

A previous study reported that the high anthocyanin content (52 mg g^{-1} extract) of black chokeberry pomace extract was responsible for inhibiting the oxidative process of rapeseed oil in $W_1/O/W_2$ emulsions (Eisinaitė et al., 2021). Similarly, dry microcapsules containing pomegranate peel extract delayed lipid oxidation of a salad dressing produced with 50 wt% peanut oil (Yang et al., 2024). The authors mentioned that the hydroxyl groups of pomegranate peel polyphenols donated hydrogen atoms to eliminate free radicals such as hydroxyl, peroxyl, superoxide, and nitric oxide, delaying the initiation or propagation of lipid oxidation. Overall, $W_1/O/W_2$ emulsions stabilized with LMPC-TA or those produced with CEB showed comparable results in reducing lipid oxidation. Therefore, these results confirm $W_1/O/W_2$ as a successful strategy to protect the oil phase against oxidation using the antioxidant potential of CBE, a rich-in-polyphenols extract from an agri-food by-product, with a LMPC-TA conjugate, an antioxidant emulsifier from an alternative protein source.

4. Conclusion

Double emulsions stabilized with mealworm protein concentrate, LMPC, or LMPC conjugated with tannic acid were successfully used to encapsulate polyphenols from beet by-product extract (CEB). The $W_1/O/W_2$ emulsions were produced using a low-energy, high-throughput technology based on dynamic membranes of tunable pore size using two different pressures (300 or 450 kPa). Emulsion fluxes higher than 100 $m^3\ h^{-1}\ m^{-2}$ and the encapsulation efficiencies of about 67 % strongly support the interest in using membrane emulsification for encapsulating polyphenols obtained from an agri-food by-product. Both lesser mealworm protein concentrates and LMPC conjugated with tannic acid successfully stabilized the O/W_2 interphase, but the protein-polyphenol conjugate was more efficient in preventing oil oxidation during storage, with a more notable effect at 25 °C. We discovered that polyphenols from beet by-product extract and LMPC-TA inhibited lipid oxidation, thereby increasing the shelf life of $W_1/O/W_2$ emulsions. Additionally, the potential of an insect protein conjugated with polyphenol in stabilizing double emulsions was shown for the first time. Therefore, our study suggests that using the powerful polyphenol-rich CEB and alternative insect proteins (LMPC-TA) is effective in $W_1/O/W_2$ emulsions by inhibiting the oxidation of Linseed oil's polyunsaturated fatty acids.

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CRediT authorship contribution statement

Callebe Camelo-Silva: Writing – original draft, Methodology, Investigation, Funding acquisition, Conceptualization. **Aurélie Ballon:** Methodology, Investigation. **Madushika K. Ranasinghe:** Methodology, Investigation. **Silvani Verruck:** Methodology, Investigation, Funding acquisition, Conceptualization. **Alan Ambrosi:** Writing – review & editing, Methodology, Investigation, Funding acquisition, Conceptualization. **Marco Di Luccio:** Writing – review & editing, Funding acquisition, Conceptualization. **Carme Güell:** Writing – review & editing, Supervision, Funding acquisition, Conceptualization. **Montserrat Ferrando:** Writing – review & editing, Funding acquisition, Conceptualization.

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.

Data availability

Data will be made available on request.

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