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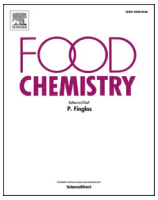

Combination of legume proteins and arabinoxylans are efficient emulsifiers to promote vitamin E bioaccessibility during digestion

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ABSTRACT

The emulsification potential of plant-based emulsifiers, that is, pea (PPI) and lentil (LPI) proteins (4%), corn arabinoxylans (CAX, 1%), and legume protein-arabinoxylan mixtures (4% proteins + 0.15 or 0.9% CAX), was evaluated by assessing: the surface tension and potential of emulsifiers, emulsifier antinutritional contents, emulsion droplet size, emulsion physical stability, and vitamin E bioaccessibility from 10% oil-in-water emulsions. Tween 80 (2%) was used as a control. All emulsions presented small droplet sizes, both fresh and upon storage, except 4% LPI + 0.9% CAX emulsion that exhibited bigger droplet sizes (d(4,3) of approximately 18.76 μm vs 0.59 μm for the control) because of droplet bridging. Vitamin E bioaccessibility from emulsions stabilized with the combination of 4% PPI and either 0.15% or 0.9% CAX (28 ± 4.48% and 28.42 ± 3.87%, respectively) was not significantly different from that of emulsions stabilized with Tween 80 (43.56 ± 3.71%), whereas vitamin E bioaccessibility from emulsions stabilized with individual emulsifiers was significantly lower.

1. Introduction

Oil-in-water (O/W) and water-in-oil emulsions are colloidal dispersions of two immiscible phases consisting of small droplets of one phase dispersed within the other (Norde, 2011). Emulsion stabilization to maintain dispersed droplets in a continuous phase requires the use of emulsifiers, which are molecules capable of adsorbing and remaining at interfaces. The search for new emulsifiers to stabilize these mixtures is a challenge for the research community because emulsions are widely used for different purposes in the food, cosmetic, and pharmaceutical industries. In the specific case of the food industry, the most used emulsifiers encompass milk proteins (β-lactoglobulins and whey protein isolates) (Liang et al., 2017), polysorbates, carboxymethylcelluloses, and carrageenans (Naimi, Viennois, Gewirtz, & Chassaing, 2021). Unfortunately, most of these emulsifiers are controversial. Evidence suggests that polysorbates, carboxymethylcelluloses, or carrageenans may be responsible for the increasing number of individuals with gastrointestinal disorders (Naimi et al., 2021; Viennois & Chassaing, 2018) because of their inflammatory effects on the gut (Viennois & Chassaing, 2021). Moreover, a growing number of consumers are pushing for plant-based emulsifiers as alternatives to animal-based emulsifiers (McClements & Grossmann, 2021; Possidónio, Prada, Graça, & Piazza, 2021). The importance of increasing knowledge on plant-based emulsifiers is also driven by the emerging need for a transition towards more sustainable foods and ingredients (Berton-Carabin & Schroën, 2019). Thus, it is crucial to identify alternative emulsifiers that can potentially replace the abovementioned emulsifiers.

Several studies reporting the emulsifying properties of a wide range of plant-based proteins, carbohydrates, and/or their mixtures can be found in the literature (Kumar, Tomar, et al., 2022). Some studies have

Abbreviations: AX, arabinoxylans; CAX, corn arabinoxylans; DMACA, *p*-dimethylaminocinnamaldehyde; d(4,3), volume-mean droplet diameter; d(3,2), surface-mean droplet diameter; GAE, gallic acid equivalent; HCL, hydrochloric acid; IFT, interfacial tension; LPI, lentil protein isolate; NaCl, sodium chloride; $NaHCO_3$, sodium bicarbonate; PPI, pea protein isolate; O/W emulsion, oil-in-water emulsion.

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evaluated the emulsification properties of arabinoxylans (Bai, Huan, Li, & McClements, 2017). Arabinoxylans (AX) are constituted of a linear backbone of xylose units linked by β1–4 bonds, with arabinose units linked to some of the xylose units (Zannini, Bravo-Núñez, Sahin, & Arendt, 2022). These polysaccharides are present in the cell walls of different vegetal tissues and are often discarded because they are mostly concentrated in the bran cell walls of grains (wheat or corn grains) (Zannini et al., 2022). Currently, only 10% of the produced wheat bran is used by the food industry: the remaining 90% is sold as animal feed or wasted (Gómez, Gutkoski, & Bravo-Núñez, 2020; Onipe, Jideani, & Beswa, 2015). Thus, the use of AX as an emulsifier constitutes an interesting way to valorize these byproducts. Additionally, AX has beneficial effects on the gut, as it displays a prebiotic effect (Gill, Rossi, Bajka, & Whelan, 2021): selective fermentation can result in specific changes in the composition and/or activity of the gastrointestinal microbiota (Gibson et al., 2010). The synergistic effect of AX and milk proteins as emulsifiers has already been addressed (Li et al., 2021; Yadav, Parris, Johnston, Onwulata, & Hicks, 2010; Zhai, Gunness, & Gidley, 2021), showing either improved droplet size and stability, or enhanced oxidation stability after AX inclusion. However, no information is available regarding the potential synergistic effects of AX and plant-based emulsifiers. To date, the most popular plant-based emulsifiers have been derived from soybean crops (soy proteins and lecithin), despite the detrimental effects of intensive soybean culture on the environment (Novelli, Caviglia, Jobbágy, & Sadras, 2023). Although efforts are being made to improve the sustainability of soybean crops, legume crops are more sustainable (Wacker & Dresbøll, 2023). The potential of lentil and pea proteins as food ingredients with emulsifying properties has been investigated recently (Funke, Boom, & Weiss, 2022; Oliete, Potin, Cases, & Saurel, 2019; Osemwota, Alashi, & Aluko, 2021). Therefore, it would be interesting to understand the behavior of such legume proteins when combined with AX, which has never been studied.

Emulsifiers affect not only the characteristics of emulsions but also nutrient assimilation by the body. For fat-soluble vitamins, the bioaccessibility from emulsions is strongly affected by the physical characteristics of the emulsions (such as droplet size or aggregation) (Tan & McClements, 2021)) and the chemical nature of the emulsifiers used (Lv, Zhang, Tan, Zhang, & McClements, 2019). The levels of micronutrients (such as vitamins or polyphenols) and antinutritional compounds (such as tannins, phytates, and saponins) associated with emulsifiers (such as legume proteins), together with the foods and polymers being consumed at the same time, either enhance or reduce vitamin absorption in the gastrointestinal tract (Antoine et al., 2021; Margier et al., 2019).

Understanding and modulating the behavior of legume proteins combined with AX as emulsifiers can generate valuable knowledge for the development of sustainable and healthy plant-based emulsions with improved functionality. Thus, the aims of this study were to i) evaluate the potential of the combination of legume proteins (lentil and pea protein isolates) and AX (from corn) as emulsifiers to stabilize O/W emulsions, and ii) address whether these new emulsifiers modulate fat-soluble vitamin bioaccessibility compared to a control emulsifier (polysorbate).

To this end, the emulsification properties of legume protein-AX mixtures at different concentrations were evaluated by assessing the surface tension and ζ-potential of the emulsifiers, emulsifier antinutritional content, emulsion droplet size, emulsion stability, and microstructure (light microscopy). The emulsions were also loaded with vitamin E to determine vitamin E bioaccessibility using an in vitro digestion system.

2. Materials and methods

2.1. Materials

Lentil protein isolate (LPI) (LTVCP-80C, 80% protein) was kindly donated by AGT Foods (Regina, SK, Canada). Pea protein isolate (PPI) (Nutralys®F85F, min 83% protein) was kindly donated by Roquette (Lestrem, France). Corn arabinoxylans (CAX) (minimum 79.3% soluble fiber) were kindly donated by AgriFiber (Mundelein, IL, USA). All analytical grade chemicals were purchased from Sigma-Aldrich (Saint-Quentin-Fallavier, France).

2.2. Tannin assay

Emulsifier tannin content was determined using the spectrophotometric method described by Antoine et al. (2022) with some modifications. Briefly, emulsifiers were suspended in Milli-Q water (1% *w/v*) and mixed for 30 min at ambient temperature and 2000 rpm. Solutions were centrifuged (10 min at 4 °C and 4400*g*). 4 mL of the supernatant were then vigorously mixed with 2 mL of a 50% methanol solution (*v*/v) and 1 mL of a 0.72% (*w/v*) *p*-dimethylaminocinnamaldehyde (DMACA) solution of 82% methanol and 18% hydrochloric acid (HCL) (*v*/v) at ambient temperature for 20 min. The absorbances of the solutions were measured at 640 nm against a reagent blank using a Shimadzu UV 8000 spectrophotometer (Shimadzu, Marne-la-Vallée, France). The reagent blank was made of 4 mL of Milli-Q water, 2 mL of a 50% methanol solution (v/v), and 1 mL of a 0.72% (w/v) DMACA solution, and was treated as the other samples prior to measurement. Samples were prepared in triplicate, and each measurement was performed in duplicate. A catechin standard curve was constructed.

2.3. Phytate assay

The phytate content of the emulsifiers was determined by the spectrophotometric method described by Antoine et al. (2022) with some modifications. Briefly, 200 mg of emulsifier powder was mixed with 10 mL of HCL (0.5 M) for 1 h at ambient temperature. The mixtures were then centrifuged for 10 min at 4600*g*, and the supernatant was recovered and 10 times diluted with Mili-Q water. A portion of 2 mL of thiocyanate-iron complex solution (0.75 μg iron(III) chloride/mL, 37.5 mg ammonium thiocyanate/mL, and 0.2% (v/v) nitric acid) was added to 1 mL of the resulting dilution, and the mixture was agitated in a water bath for 2 h and 30 min at 40 °C. The tubes were then cooled for 30 min and centrifuged for 5 min at 4600*g* to remove residual iron. The absorbance of the supernatant was measured at 480 nm against a reagent blank using a Shimadzu UV 8000 spectrophotometer (Shimadzu, Marne-la-Vallée, France). The reagent blank was made of 1 mL Milli-Q water and 2 mL thiocyanate-iron complex solution and was treated as the other samples prior to measurement. Samples were prepared in triplicate, and each measurement was performed in duplicate. A standard curve was constructed using phytic acid.

2.4. Saponin assay

Saponin content was determined using a colorimetric vanillin-sulfuric acid assay, following the methodology described by Bravo-Núñez, Golding, McGhie, Gómez, and Matía-Merino (2019) with some modifications. Briefly, 0.25 g of each sample were combined with 2.5 mL of aqueous methanol (80%) and left under continuous agitation for 24 h. Then, samples were submitted to centrifugation 10 min at 4 °C at 4600*g*. The supernatant was collected and stored at 4 °C and extraction was repeated twice by adding 0.25 mL of methanol to the lower phase. The supernatants of the three extracts were mixed and used for saponin determination. The supernatant (0.25 mL) was then transferred to a glass tube. Then 0.25 mL of 8% vanillin reagent (8% vanillin in pure ethanol, *w/v*) and 2.5 mL of 72% sulfuric acid were added to the tubes. The solutions were vortexed for 30 s and the tubes were incubated at 63 °C for 10 min. After incubation, the samples were cooled for 5 min in cold water, and the absorbance was measured at 544 nm against the reagent blank using a Shimadzu UV 8000 spectrophotometer (Shimadzu, Marne-la-Vallée, France). The reagent blank was made of 0.25 mL of aqueous methanol (80%), 0.25 mL of 8% vanillin reagent, and 2.5

mL of 72% sulfuric acid and was treated as the other samples prior to measurement. Samples were prepared in triplicate, and each measurement was performed in duplicate. A standard curve was constructed using soy saponin I.

2.5. Phenolic content assay

PPI (4% *w*/w), LPI (4% w/w), and CAX (1% w/w) aqueous solutions were prepared by dissolving powders in Milli-Q water. The samples were magnetically stirred (400 rpm) overnight for hydration. The total phenolic content of the samples was determined using the Folin-Ciocalteu assay. Briefly, 250 μL of each solution was mixed with 250 μL of the Folin–Ciocalteu reagent. Then, 5 mL of a saturated sodium carbonate solution (7.5%) was added to the mixture, and the mixture was vigorously mixed. The absorbance of the solution was measured at 725 nm after 1 h in the dark using a V-670 spectrophotometer (Jasco, Tokyo, Japan). Quantification was based on a standard curve for gallic acid. Samples were prepared in triplicate, and each measurement was performed in duplicate. The results were expressed as milligrams of gallic acid equivalent (GAE) per milliliter of sample (mg GAE/mL).

2.6. Interfacial tension of emulsifiers

PPI (0.04% *w*/w), LPI (0.04% w/w), and CAX (0.01% w/w) aqueous solutions were prepared by dissolving powders in Milli-Q water. The samples were magnetically stirred (400 rpm) overnight for hydration. The preliminary aqueous solutions were further diluted (1/10, *v*/v) with Milli-Q water and stirred (400 rpm) for 30 min, followed by 15 min of sonication in a water bath and another 15 min of stirring (400 rpm). Thus, PPI aqueous solution (0.004% w/w), LPI aqueous solution (0.004% w/w), and CAX aqueous solution (0.001% w/w) were prepared. In addition, blends of PPI or LPI (0.004% w/w) with CAX at two concentrations (0.0009 or 0.00015% w/w) were also prepared from preliminary solutions. A drop shape analyzer DSA25 (KRÜSS, Hamburg, Germany) was used to evaluate the changes in interfacial tension (IFT; mN/m) caused by PPI, LPI, CAX, or their blends. A droplet of medium-chain triglyceride oil was formed at the tip of a U-shaped needle, whereas the aqueous protein solutions were placed in a cuvette. Samples were prepared in triplicate, and each measurement was performed in duplicate. Pendant drop measurements recording the changes in the IFT were performed in duplicate for 2 h.

2.7. ζ-potential of emulsifiers

For ζ-potential determination, 4% PPI (*w*/w), 4% LPI (w/w), 1% CAX (w/w), 4% PPI + 0.9% CAX (w/w), 4% PPI + 0.15% CAX (w/w), 4% LPI + 0.9% CAX (w/w), and 4%LPI + 0.15% CAX (w/w) aqueous solutions were prepared with milli-Q water. The aqueous solution was then diluted to 1/10 (*v*/v). ζ-potential of resulting solutions was measured using a Zetasizer Nano ZS instrument (Malvern Instruments Ltd., Worcestershire, UK) at 20 °C using a refractive index of 1.45. Each sample was analyzed in duplicate. Three independent batches were analyzed.

2.8. Emulsion preparation

O/W emulsions were prepared by emulsifying olive oil at 10% (w/w) and different PPI, LPI, and CAX aqueous solutions elaborated with Mili-Q water (4% PPI (w/w), 4% LPI (w/w), 1% CAX (w/w), 4% PPI + 0.9% CAX (w/w), 4% PPI + 0.15% CAX (w/w), 4% LPI + 0.9% CAX (w/w), and 4% LPI + 0.15% CAX (w/w) solutions). Briefly, oil + aqueous solutions were pre-homogenized for 2 min at 12000 rpm with an Ultra-Turrax® (Ika, Staufen, Germany). Coarse emulsions were immediately homogenized with an Ultrasound Brandson 450 version 01.02 (Brookfield, Connecticut, USA) equipped with a 1/8″ diameter tapered probe at 70% amplitude during 6 min. During ultrasound homogenization, cold ethanol was placed around the tubes to avoid an increase in temperature. Two drops of sodium azide solution (1%, *w*/*v*) were added to the freshly homogenized emulsions as antimicrobial agents during stability tests. Emulsions were stored at 4 °C between measurements. To determine the droplet size and physical stability, the emulsions were prepared in triplicate, and each emulsion was measured in duplicate.

When vitamin E was included in the emulsions, an appropriate volume of vitamin E (α-tocopherol) stock solution in HPLC grade ethanol was transferred to a glass tube, the solvent was evaporated using nitrogen and the dried residue was solubilized in olive oil to achieve an α-tocopherol concentration of 0.18 mg α-tocopherol /mL olive oil. The same protocol was followed to elaborate the emulsions, thereby protecting them from light during all steps to avoid α-tocopherol degradation.

2.9. Droplet size measurement

The droplet size distribution of the O/W emulsions was determined by laser light scattering using a Malvern MasterSizer Micro V2.15 (Malvern Instruments Ltd., 205 Worcestershire, UK). Milli-Q water was used as the dispersant and the relative refractive index (N) of the emulsion was 1.105, that is, the ratio of the refractive index of olive oil (1.470) to that of the aqueous medium (1.33). The size of the emulsion droplets was analyzed on the same day of preparation and on subsequent days (1st, 2nd, 7th, 14th' and 21st day). Emulsions were stored at 4 °C and were always gently mixed before sampling to obtain representative measurements. The volume-mean droplet diameter (d(4,3)) and surface-mean droplet diameter (d(3,2)) were assayed. Emulsions were prepared in triplicate, and each emulsion was measured in duplicate.

2.10. Emulsion microstructure

A Zeiss Axio Imager M1 microscope (Carl Zeiss, Oberkochen, Germany) was used to visualize the microstructure of the emulsions. For light microscopy, an aliquot of emulsion sample, diluted five times, was placed on a microscope slide. A coverslip was placed on the top of each well to ensure that no air bubbles were trapped. Images were captured at 10× and 100× magnifications in duplicate.

2.11. In vitro digestion of emulsions

The in vitro digestion was performed as described previously (Malapert et al., 2018). Briefly, 8 mL of 0.9% NaCl was added to a meal composed of 1.67 g of pureed potatoes and 0.3 g of fried minced beef. The mixture was dispersed for 2 min with an Ultra-Turrax® (Ika) at 12000 rpm, and 1 g of the vitamin-loaded emulsion and 0.05 g of olive oil were added. A control emulsion was elaborated with an emulsifying solution of Tween 80 2% (*w*/w). For the control meal, water (1 g) and 0.15 g of extra virgin olive oil were added to match the same olive oil concentration as in the rest of the samples. Next, 0.63 mL of artificial saliva (pH 7) was added to the mixture. The samples were incubated for 10 min at 37 °C in a shaking incubator to simulate salivary flow. Subsequently, the pH was adjusted to 4.00 ± 0.02 with 1 M HCl and porcine pepsin (0.5 mL, 40 mg/mL in 0.1 M HCl) was added. The samples were incubated at 37 °C for 30 min to simulate the gastric step. The pH of the samples was then raised to 6.00 ± 0.02 with 0.9 M sodium bicarbonate ($NaHCO_3$) and 2.25 mL pancreatin (3 mg/mL in 0.1 M trisodium citrate pH 6) and 1 mL of porcine bile extract (127 mg/mL in 0.1 M trisodium citrate pH 6) was added. The samples were subsequently incubated in a shaking incubator at 37 °C for 30 min to simulate the duodenal step and to complete the digestion process. The final digesta was centrifuged (2000*g* for 1 h 12 min at 10 °C) and the supernatant was filtered with serial 0.8 μm and 0.2 μm filters (Millipore, Burlington, MA, USA). All analyses were performed in quadruplicate. Aliquots of digesta (before centrifugation) and micelles (after centrifugation and filtration) were frozen at −80 °C until use. The bioaccessibility was calculated using the following equation:

$$\text{Bioaccesibility (\%)} = \frac{\frac{\text{ng α−tocopherol}}{\text{g micelles}}}{\frac{\text{ng α−tocopherol}}{\text{g digesta}}} * \frac{\text{g micelles}}{\text{g digesta}} * 100$$

2.12. Vitamin E extraction and chromatographic analysis

To evaluate vitamin E content, vitamin E was extracted from either 500 μL of emulsifier solution, 250 μL of digesta, and 500 μL micelles. Milli-Q water was added to the samples to reach a final volume of 500 μL. The same volume of internal standard solution (0.01 ng retinyl acetate/μL ethanol), and 2 mL of hexane was added to glass tubes and samples were vigorously mixed for 5 min with a vortex at ambient temperature. Next, samples were centrifuged (10 min 4 °C, 1363g) and the upper phases were collected in hemolysis tubes. An additional 2 mL of hexane was added to the glass tubes and the process was repeated. The second upper phases were collected, pooled to the first ones, and evaporated to dry using nitrogen. The dried extracts were dissolved in 200 μL of pure methanol. A volume of 5–180 μL was used for HPLC analysis with a 250 × 4.6 nm RP C18, 5 μm Zorbax column (Agilent Technologies, Montpellier, France) maintained at a constant temperature (35 °C) and a guard column with a mobile phase of 100% methanol (flow rate = 1.5 mL/min). The HPLC system comprised a Dionex separation module (P680 HPLC Pump and ASI-100 Automated Sample Injector, Dionex, Aix-en-Provence, France), a Dionex UVD340U photodiode array detector (vitamin E detection at 292 nm and retinyl acetate detection at 325 nm), and a JASCO fluorimetric detector (JASCO, Nantes, France). For fluorimetric analysis, α-tocopherol and γ-tocopherol were detected at 325 nm after light excitation at 292 nm. The system was controlled by Chromeleon software (v6.50 SP4 Build 1000, Dionex). α-tocopherol and γ-tocopherol calibration curves allowed to quantify vitamin E by comparing the sample peak area with standard reference curves.

2.13. Statistical analysis

Data were expressed as mean ± SEM. Statistical analyses were performed using GraphPad Prism software, version 8.4.3 (GraphPad Software, San Diego, California, U.S.A.). Prior to one-way ANOVA, data were tested for normality using Shapiro-Wilk test) The distribution of all the dependent variables was normal. Tukey's test was used as a post hoc test for pairwise comparisons. Values of $p \leq 0.05$ were considered significant.

3. Results and discussion

3.1. Emulsifier composition

Legume proteins and CAX, even when purified, can still be associated with antinutritional compounds and/or various bioactive compounds. Thus, we first evaluated the antinutritional and micronutrient contents of the emulsifiers, as the presence of these compounds can have an important effect on vitamin bioaccessibility, as discussed later in this manuscript.

The antinutritional compound contents (tannins, phytates, and saponins) of the tested emulsifiers are shown in Fig. 1. All the emulsifiers had a low tannin content. However, CAX had a significantly higher tannin content than PPI and LPI, and PPI had a significantly higher tannin content than LPI. Because the tested emulsifiers were originally derived from grains, the presence of phytates was expected in all emulsifiers. Indeed, phytates represent between 1 and 5% (dry weight basis) of most oilseeds, legumes, cereals, nuts, and pollen (Graf, 1990). As anticipated, phytates were the most abundant antinutritional compounds for all emulsifiers. No significant differences between the emulsifiers were found among the groups. Finally, no significant difference was found between PPI and LPI with respect to saponin content. Conversely, CAX contains a significantly lower concentration of saponins than LPI. The presence of saponins in legumes is consistent with previous data (Kumar, Basu, et al., 2022; Margier et al., 2018).

The phenolic contents of the emulsifier solutions revealed no significant differences between the samples (Supplementary Table S1). However, concentrations of LPI or PPI in solutions were higher than those of CAX (4% versus 1%, *w*/w), indicating that the phenolic content of CAX was higher than that of PPI and LPI, in agreement with the literature (Boye et al., 2010).

Finally, the vitamin E content of the emulsifiers (CAX, PPI, and LPI) was assessed. No α-tocopherol was found in any of the emulsifiers. PPI and LPI presented traces of γ-tocopherol (~226 and ~22.88 μmol/ g emulsifier respectively). No γ-tocopherol was found in CAX.

3.2. Emulsifier physicochemical characteristics

To evaluate emulsifier and emulsion characteristics, we chose to work with either 4% LPI/ PPI, 1% CAX solutions, or combinations of 4% LPI/ PPI with either 0.15 or 0.9% CAX. The percentage of emulsifiers was chosen after preliminary tests (different concentrations of legume proteins ranging 2–4%, concentrations of CAX up to 1%, and homogenization times ranging 3–6 min were tested, data not shown) and according to previous reports (Yadav et al., 2010, Burger & Zhang, 2019).

The emulsifying potential of polymers can be envisaged by

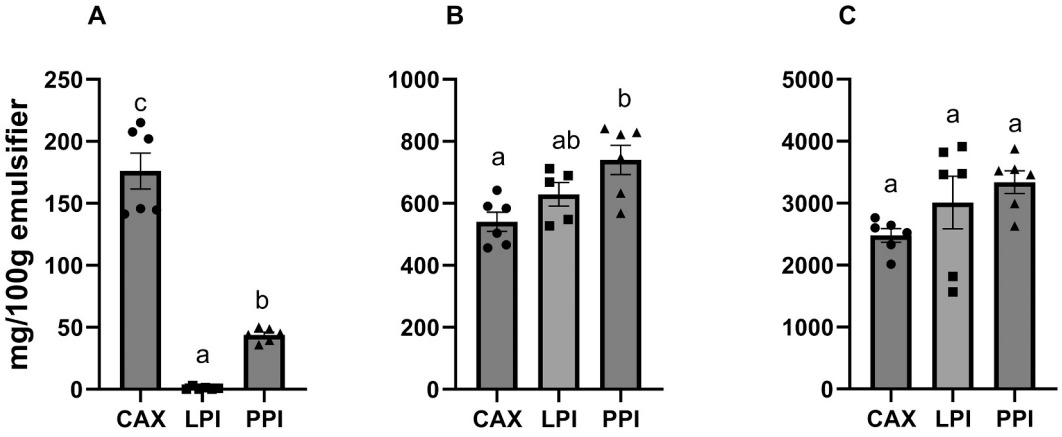

Fig. 1. A) Tannin, B) saponin, and C) phytate content of corn arabinoxylans (CAX), lentil protein isolate (LPI), and pea protein isolate (PPI). Within the same antinutritional compound, means not sharing a letter are significantly different ($p \leq 0.05$).

determining i) their interfacial activity, as it plays an important role in determining their ability to form and stabilize emulsions (Schubert & Engel, 2004), and ii) their ζ-potential and interfacial tension (IFT), which enables the detection of the potential surface-activity of food emulsifiers.

It was first observed that all emulsifier solutions presented a negative ζ-potential ≤ -30 mV, except the 1% CAX solution that presented a slightly higher ζ-potential (-27.16 ± 1.23 mV) (Supplementary Table S1). When combined, the presence of CAX did not significantly modify the ζ-potential of LPI or PPI. Lower ζ-potentials were previously reported for emulsifier solutions containing PPI. According to the available literature, solutions and emulsions having a ζ-potential lower than −30 mV tends to be electrostatically stable, whereas those with an electrical charge above −30 mV are more likely to flocculate/coagulate (Intarasirisawat, Benjakul, & Visessanguan, 2014).

For all emulsifier solutions containing LPI or PPI, the IFT plots (Fig. 2) exhibited an initial rapid decrease, which could be attributed to the adsorption of legume proteins at the interface. According to Beverung, Radke, and Blanch (1999), a faster adsorption rate is related to higher hydrophobicity of the emulsifiers. After that, the change of the curve leveled off probably due to the adsorption and covering of the emulsifiers at the interface (at $t = 115$ min PPI and LPI solutions presented IFT values of 18.85 ± 1.13 and 23.57 ± 0.81 mN/m, respectively (Supplementary Table S1). CAX did not significantly reduce IFT when compared to the values obtained with Milli-Q water (at t = 115 min CAX solution and Milli-Q water presented IFT values of 35.84 ± 1.60 and 36.71 ± 0.59 mN/m respectively). This lack of decrease in IFT by the CAX solution could be due to the dilution level, since, as presented later in the manuscript, CAX has an emulsifying capacity. The combination of CAX with PPI or LPI did not significantly increase the IFT obtained with either PPI or LPI alone. However, we observed that mixtures containing LPI and CAX at both levels presented significantly ($p < 0.02$) higher IFT than that of PPI (at t = 115 min, diluted 4% LPI + 0.15% CAX, 4% LPI + 0.9% CAX and 4% PPI solutions presented IFT values of 25.61 ± 0.71, 24.47 ± 0.02, and 18.85 ± 1.13 mN/m, respectively). This is likely related to the different IFT of PPI and LPI, which are lower for the PPI solutions.

3.3. Emulsion droplet size

The emulsion droplet size depends on the emulsifier type and concentration, as well as the oil and water volume fractions. Characterization of droplet size can help predict and optimize the behavior of emulsions during production and application (Xia, Xue, & Wei, 2021). We reported two different droplet size measures: i) d(3,2), which considers the average surface area of the droplets and is most sensitive to the presence of fine particulates in the size distribution, and ii) d(4,3), which considers the average volume and is most sensitive to the presence of large particulates in the size distribution. Both measurements provide valuable information regarding the characteristics of emulsions and have been widely reported in the literature (Mcclements, 2007). The mean droplet sizes of the emulsions are shown in Figs. 3A and B.

The use of 1% CAX resulted in small droplet sizes (d(4,3) of 0.93 ± 0.05 μm), like those observed for 2% Tween 80 emulsions (d(4,3) of 0.59 ± 0.01 μm). This was unexpected, considering the high IFT of CAX (Fig. 2A). The emulsification potential of AX from different sources has been reported previously, but with larger droplet size distributions (Bai et al., 2017; Kale, Yadav, Chau, & Jr, 2018). Bai et al. (2017) evaluated the effect of AX concentration on droplet size distribution and reported a decrease in droplet size with increasing AX concentration (tested concentrations from 0.1 to 5% *w*/w). At a concentration of 5% of AX, authors observed a mean droplet size of ~5–6 μm (depending on the used oil), and based on their droplet size distribution graphs, we estimate that their mean droplet size for 1% concentration could be ~ 10 μm. A previous study by Yadav et al. (2010) reported the droplet size of emulsions stabilized with CAX. Their results are consistent with our results (~1 μm), although particle size increased upon storage (~6 μm). This difference may be related to the emulsification process conditions,

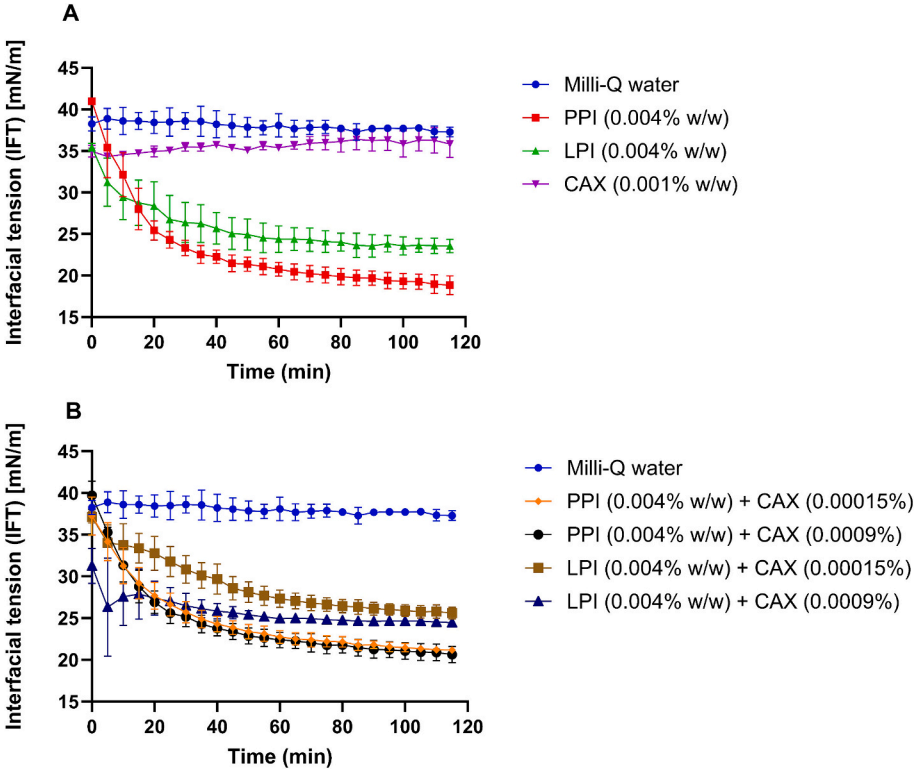

Fig. 2. Interfacial tension of emulsifier solutions of A) 0.001% CAX, 0.004% PPI, and 0.004% LPI and B) 0.004% PPI + 0.00015% CAX, 0.004% PPI + 0.0009% CAX, 0.004% L PI + 0.00015% CAX, and 0.004% LPI + 0.0009% CAX.

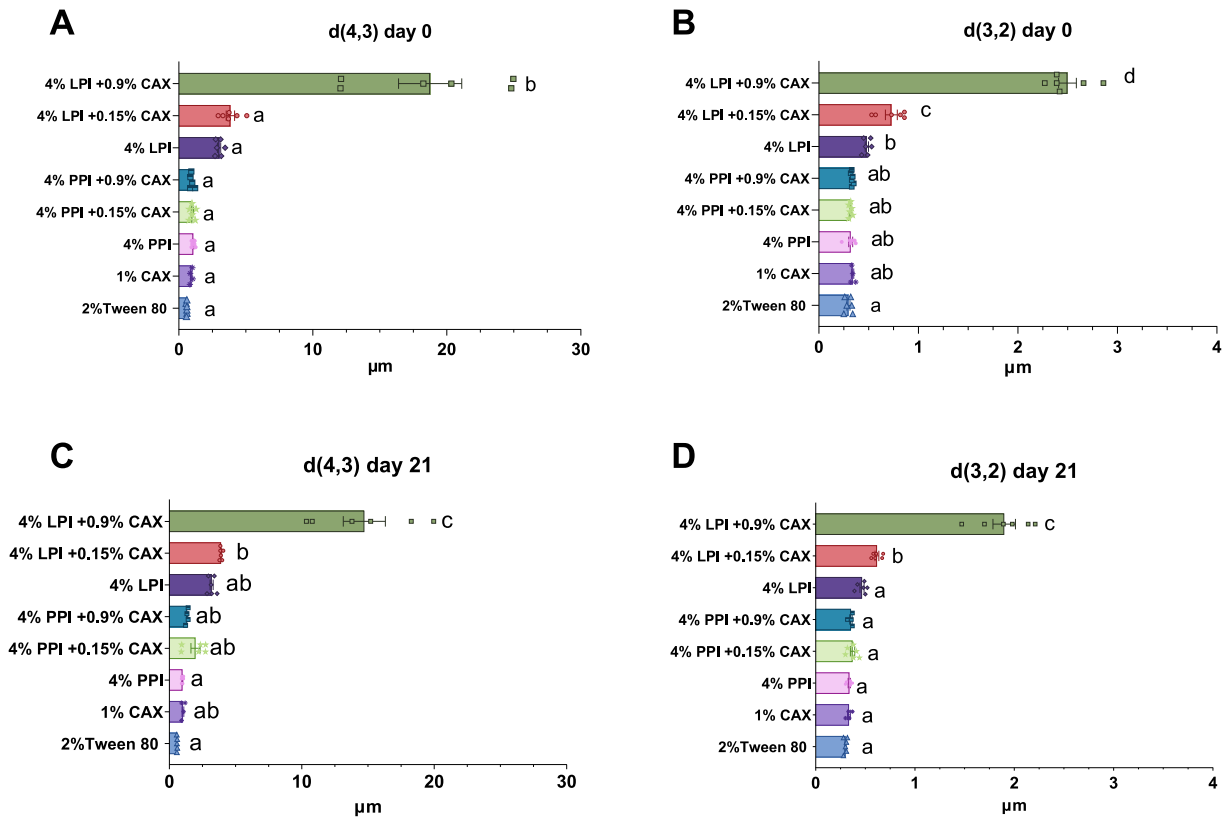

Fig. 3. d(4,3) and d(3,2) of 10% oil-in-water fresh emulsions (A and B) and after 21 d of storage (C and D). A different statistical analysis was applied each day and for each particle size. Within the same group, means not sharing a letter are significantly different ($p \leq 0.05$).

purity, structure (arabinose/xylose ratio), and the AX source. AX branching (higher arabinose/xylose ratio) improved the emulsifying properties of AX (Yadav & Hicks, 2018). As a matter of fact, we also tested the potential emulsifying properties of commercial wheat AX with lower purity than our CAX, and these wheat AX resulted in highly unstable emulsions (d(4,3) of ~27.7 µm when using 1% wheat AX versus d(4,3) of ~0.93 um when using 1% CAX, together with the presence of free oil and instant phase separation, data not shown). Poor emulsification properties of wheat AX compared to those of other sources have also been reported by Yadav and Hicks (2018). The differences between our results and previously published data cannot be attributed to the potentially different molecular weights of AX, as the molecular weight does not modulate the emulsifying properties of AX (Kale et al., 2018). Fig. 4B revealed the presence of bridging phenomena between droplets in 1% CAX emulsions, which could be caused by attractive electrostatic interactions between CAX polymers absorbed to the interface of different oil droplets (Walstra, 2003) and the higher ζ-potential of 1% CAX solution (−27.16 ± 1.23 mV, Supplementary Table S1) compared

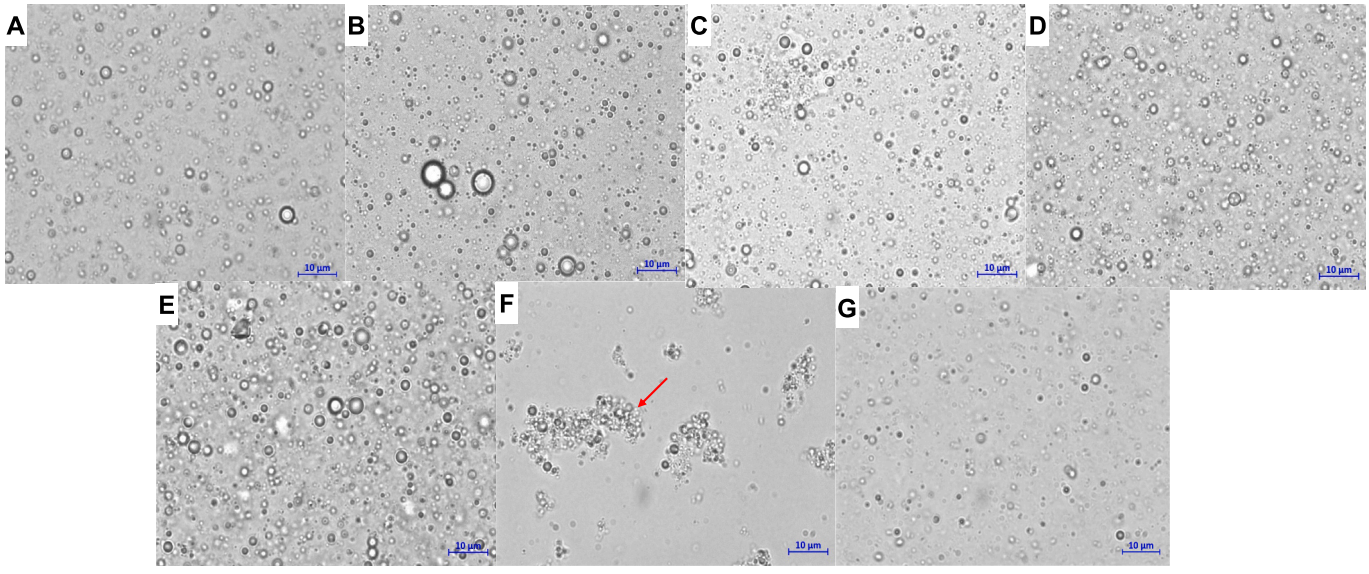

Fig. 4. Light microscopy images of 10% oil-in-water emulsions stabilized with A) 2% Tween 80 B) 1% CAX C) 4% PPI, D) 4% PPI + 0.9% CAX, E) 4% LPI, F) 4% LPI + 0.9% CAX, and G) 4% LPI + 0.9% CAX with 1% SDS.

to the other emulsifying solutions.

Emulsions stabilized with LPI or PPI also presented small droplet sizes, and although no significant differences were reported between them, smaller droplet sizes were observed for emulsions stabilized with 4% PPI than for those stabilized with 4% LPI (d(4,3) of approximately 1.09 vs 3.01 μm). When compared with the control emulsion, only 4% LPI emulsion presented a significantly higher d(3,2) droplet size (0.48 ± 0.02 vs 0.3 ± 0.02 μm, Fig. 2B). Small particle sizes of emulsions stabilized with pea protein have been reported elsewhere (Oliete et al., 2019), and the potential of PPI as an emulsifier is well acknowledged (see Burger & Zhang, 2019). The emulsification properties of LPI have also been previously reported, but to a lesser extent than those of pea proteins. As for CAX, we found smaller droplet sizes for emulsions stabilized with LPI than those reported elsewhere (d(3,2) ~ 6–10 μm) (Funke et al., 2022). Such differences may be due to the emulsification process, as the above-mentioned studies used a less intense emulsification process prior to emulsion characterization. This may also be related to the fine structure and purity of the protein (Avramenko, Low, & Nickerson, 2013), as well as the method of extraction/purification of proteins.

When looking at the microstructure of these emulsions (Fig. 4), it seems that the significant increase in droplet size (d(3,2)) of 4% LPI emulsion compared to both the control and 4% PPI emulsions (Figs. 4C and E) could be due to i) more extensive bridging-flocculation phenomenon between oil droplets and ii) some coalescence due to a weaker LPI adsorption at the interface. LPI may be incapable of preventing some oil leakage and migration from smaller to larger droplets, leading to droplet growth. Primozic, Duchek, Nickerson, and Ghosh (2017) already reported bridging-flocculation of emulsions stabilized with different concentrations (0.5–5%) of LPI. The stronger bridging-flocculation phenomenon of 4% LPI vs. 4% PPI may be related to the higher IFT of 4% LPI solutions (see 3.2. section). It could be that, owing to a lack of sufficient emulsifying capacity of 4% LPI solution for the available oil, the individual LPI molecules stabilized several droplets at the same time, favoring the observed bridging-flocculation phenomena. However, this phenomenon did not influence the droplet stability over time (see 3.4. section). The combination of PPI or LPI with different concentrations of CAX (0.15 and 0.9%) bearded to an increase in droplet size, although only the combination of 4% LPI + 0.9% CAX significantly increased both d(4,3) and d(3,2) droplet sizes of fresh emulsions compared to the control (Fig. 3A). The microstructure of this emulsion (Fig. 4F) revealed that the abrupt increase in particle size of 4% LPI + 0.9% CAX emulsion compared with the other emulsions was not due to much larger droplet size, but due to extensive bridging flocculation phenomena between droplets. This was likely due to interactions between LPI and CAX, as emulsions stabilized either with 4% LPI or 1% CAX alone did not present this extensive flocculation at the microscopic level.

To understand the mechanism underlying this flocculation, a freshly prepared 4% LPI + 0.9% CAX emulsion was mixed with a 2% SDS solution at a ratio of 1:1. An abrupt decrease in particle size was observed: d(4,3) of approximately 1.71 μm when SDS was added vs 18.76 μm for the original emulsion because droplet flocs/aggregates were dispersed (Fig. 4G). This proves the occurrence of the bridging flocculation phenomenon. Polysaccharides (such as CAX) have a strong affinity for droplet interfaces (Bravo-Núñez et al., 2019; Walstra, 2003), generally because of attractive electrostatic interactions. We also tested the potential emulsifying properties of a combination of legume proteins with commercial wheat AX of lower purity than that of CAX, which resulted in unstable emulsions with free oil (data not shown).

No other studies have evaluated the synergistic effects of legume proteins and AX as emulsifiers. To date, only studies evaluating the synergy between AX and animal proteins are available. Consistent with our findings, Li et al. (2021) observed an increase in droplet size when animal proteins (whey protein isolates) were combined with AX from different sources. Yadav et al. (2010) evaluated the conjugation of whey protein with CCAX and showed that conjugation by exposing protein-CAX mixtures to high temperature and humidity decreased the particle size of emulsions when compared with emulsions stabilized with either whey proteins or CAX.

3.4. Emulsion stability over time

All the emulsions were stable over time (21 days). Time did not increase the droplet size, and when analyzing particle size during 21 days of storage, a trend similar to that observed for fresh emulsions was reported for both d(4,3) and d(3,2) (Fig. 3). The only difference when comparing fresh and stored emulsions was that after 21 days of storage, emulsions containing LPI and CAX (4% LPI + 0.15% CAX and 4% LPI + 0.9% CAX) presented a significantly higher d(4,3) and d(3,2) values than the rest of emulsions. For fresh emulsions, significant differences between the control and other emulsions were observed for d(3,2) only.

Fig. 5 shows a visual separation of the selected emulsions. No phase separation was observed for emulsions stabilized with either 2% Tween 80 or 4% LPI. Significant phase separation that did not affect droplet size upon storage (Fig. 3) was observed for emulsions stabilized with 1% CAX and with 4% PPI+ 0.9% CAX (Fig. 5B and F). A similar trend was observed for the 4% PPI+ 0.15% CAX emulsion (data not shown). Phase separation was more important for emulsions stabilized only with 1% CAX (Fig. 5B) than for those stabilized with a combination of 4% PPI and 0.9% CAX (Fig. 5F), considering that CAX levels were similar between these emulsions (1% vs. 0.9%). As shown in Supplementary Table S1 and Fig. 2, ζ-potential and IFT of 1% CAX solution were significantly higher than those of 4% LPI + 0.9% CAX solution, which could partially justify the phase separation of emulsion stabilized with only 1% CAX. Surprisingly, emulsions stabilized with a combination of 4% LPI and up to 0.9% CAX presented a low phase separation. We suspect that this could be related to the emulsion viscosity, but this hypothesis should be further confirmed. Differences in phase separation at similar CAX concentrations suggest that the presence of legume proteins, especially LPI, may reduce the attractive forces between oil droplets. However, when taking into consideration droplet size results from MasterSizer measurements, it seems that attractive forces between oil droplets of O/W emulsions stabilized with a combination of 4% LPI and 0.9% CAX are stronger than those stabilized only with 1% CAX. This hypothesis is built on the fact that the dispersion force of the MasterSizer was not able to break down the oil droplet aggregates of emulsion stabilized with 4% LPI + 0.9% CAX solution, giving high d(4,3) and d(3,2) values for this emulsion. On the contrary, this same dispersion force was able to break forces attracting droplets of emulsions stabilized with 1% CAX, as the droplet sizes given by the MasterSizer (Figs. 3A and B) correspond to individual droplet sizes observed under the microscope (Fig. 4B) (droplet size of 4% LPI + 0.9% CAX << droplet size 1% CAX emulsion). A better understanding of legume protein-AX molecular-level interactions is required to fully understand the mechanism underlying the emulsion stabilization process.

3.5. Vitamin E in vitro bioaccessibility

To determine the fat-soluble vitamin bioaccessibility, an in vitro digestion method, including a control meal, was used. This specific method has been widely used by our research group (Antoine et al., 2021, 2022; Margier et al., 2019) because of its ability to predict the potential in vivo bioavailability of fat-soluble vitamins (Reboul et al., 2006).

The highest bioaccessibility was achieved with an emulsion stabilized with 2% Tween 80 (Fig. 6). The outstanding performance of this emulsifier in delivering liposoluble micronutrients has been well reported in the literature (Yang & McClements, 2013); however, its possible inflammatory effect is a drawback towards its use (Naimi et al., 2021)). Emulsions stabilized with 1% CAX presented the lowest performance regarding in vitro α-tocopherol bioaccessibility, followed by emulsions stabilized with LPI or PPI alone and mixtures of 4% LPI with

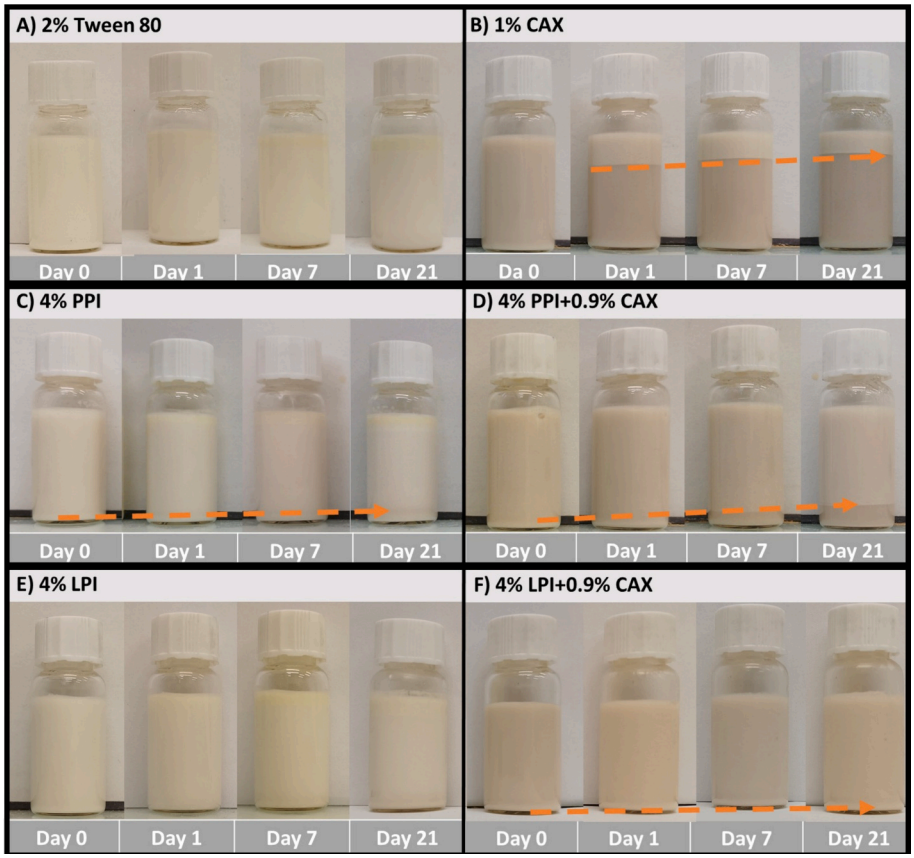

Fig. 5. Visual separation upon storage of 10% oil-in-water emulsions stabilized with A) 2% Tween 80 B) 1% CAX, C) 4% PPI, D) 4% PPI + 0.9% CAX, E) 4% LPI, and F) 4% LPI + 0.9% CAX.

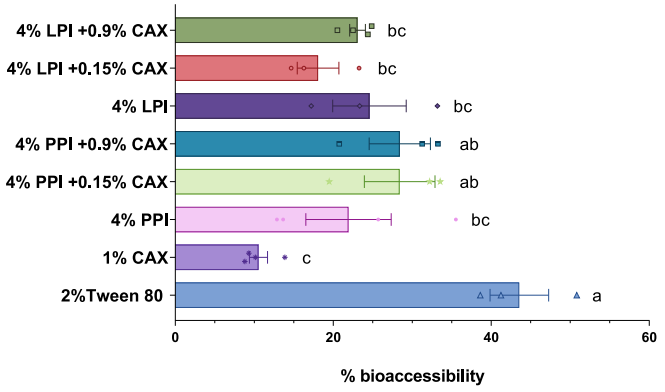

Fig. 6. In vitro α-tocopherol bioaccessibility from 10% oil-in-water emulsions. Means not sharing a letter are significantly different ($p \leq 0.05$).

both levels of CAX. As previously stated, when comparing the anti-nutritional compound profiles of LPI and PPI with those of CAX, CAX presented a significantly higher level of tannins (Fig. 1), and tannins may interfere with the bioaccessibility of vitamin E (Antoine et al., 2022). The low vitamin E bioaccessibility when using CAX as the only emulsifier can also be explained by the lack of digestibility of AX in the small intestine (Zhang et al., 2023), preventing the encapsulated vitamin from being transferred into mixed micelles, which are vehicles required for optimal absorption by enterocytes. A moderate detrimental effect of different fibers (cellulose, pectin, guar, and alginate) on vitamin E bioaccessibility has also been previously reported (Margier et al., 2019). Fibers encompass a wide number of polysaccharides with different structures and functionalities (Gill et al., 2021). Therefore, it is important to individually evaluate their impact on bioaccessibility. To the best of our knowledge, this is the first study to evaluate the effects of AX on vitamin E bioaccessibility.

The lower bioaccessibility of LPI or PPI emulsions compared to that of Tween 80 emulsions (Fig. 6) can also be linked to the LPI/PPI content of antinutritional compounds such as tannins, saponins, and phytates (Antoine et al., 2022; Margier et al., 2019).

Emulsions stabilized with mixtures of 4% LPI and CAX (both 0.15 and 0.9% *w*/w) also showed significantly lower vitamin E bioaccessibility than that of the 2% Tween 80 emulsion. Interestingly, emulsions stabilized with mixtures of 4%PPI and CAX (both 0.15 and 0.9% w/w) were the only emulsions among the tested samples that showed bioaccessibilities (28.41 ± 4.48 and 28.42 ± 3.88%, respectively) that were not significantly different from that of emulsions stabilized with 2% Tween 80 (43.56 ± 3.71%) (Fig. 6). Notably, this suggests that the detrimental effect of individual polymers can be overcome by their combination (bioaccessibilities of 1% CAX and 4% PPI alone were 10.54 ± 1.15 and 21.94 ± 5.40%, respectively). This may be because of the higher phenolic and γ-tocopherol contents of the solutions containing both PPI and CAX. The presence of these antioxidants may positively impact α-tocopherol bioaccessibility by preventing α-tocopherol degradation during its transfer from the emulsion to the mixed micelles. We observed a higher concentration of α-tocopherol in the micellar phases obtained from the digestion of the 4% PPI + 0.15% CAX emulsion and of the 4% PPI + 0.9% CAX emulsion (0.42 ± 0.09 and 0.42 ± 0.08 ng/μL micelles, respectively) than in the micelles obtained from the digestions of 1% CAX and 4% PPI alone (0.14 ± 0.02 ng/μL micelles and 0.32 ± 0.02 ng/μL micelles, respectively). Because of the health benefits of AX, the fact that its individual detrimental impact on vitamin bioaccessibility can be overcome in the presence of legume proteins is interesting, as it opens the door towards their inclusion in

food matrices rich in vitamins without negatively affecting their absorption. However, further studies are required to confirm this positive effect.

4. Conclusion

Collectively, the present study showed that the combination of pea protein isolate and corn arabinoxylans as emulsifiers results in emulsions with small droplet sizes that promote α-tocopherol in vitro bioaccessibility at the same level as emulsions stabilized with the polysorbate Tween 80.

This study contributes to the development of healthy and sustainable food products (beverages or sauces) that can be used as successful delivery systems for fat-soluble micronutrients, such as vitamin E. Further research is required to confirm these results in vivo before developing promising systems for application.

CRediT authorship contribution statement

Ángela Bravo-Núñez: Writing – review & editing, Writing – original draft, Visualization, Validation, Methodology, Investigation, Funding acquisition, Conceptualization. **Laura Salvia-Trujillo:** Writing – review & editing, Methodology, Conceptualization. **Charlotte Halimi:** Methodology. **Olga Martín-Belloso:** Writing – review & editing, Conceptualization. **Emmanuelle Reboul:** Writing – review & editing, Writing – original draft, Validation, Resources, Methodology, 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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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.foodchem.2024.139820.

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