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Stability of corn and olive oil-in-water emulsions supplemented with ethanol-treated rapeseed meal protein isolate

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Abstract

Rapeseed meal was treated with 70% ethanol to reduce anti-nutrient compounds, and used in the preparation of a protein isolate. The ethanol- treated rapeseed meal protein isolate (ERPI) was produced by alkaline extraction of proteins (pH 12) followed by isoelectric precipitation at pH 4.5. The aim of the present work was to evaluate the potential of ERPI as an emulsifying agent in corn and olive oil-in-water emulsions under slightly acidic conditions (pH 6). A total of nine emulsions for each type of oil were prepared to assess the significant effect of two variables, oil (5, 10, and 15% w/w) and ERPI protein concentrations (0.25, 0.5, and 1.0% w/w), on emulsion stability. The initial stability was evaluated by Gibbs free energy (ΔG) and lipid particle size distribution, while the dynamics of emulsion stability was investigated along 7 d by turbidity measurement. The increase in concentration of both types of oil positively influenced initial stability of the emulsions as indicated by ΔG. At each oil concentration, the three ERPI supplementation levels resulted in significant differences in ΔG. While in all olive oil-in-water emulsions, the highest initial stability was achieved by the addition of 0.25% ERPI; and in corn oil-in-water emulsions, lower ΔG values were achieved by supplementing either with 0.5 or 1.0% ERPI. With a few exceptions, there was an agreement between Gibbs free energy and microstructural characteristics of the emulsions. With a reduction in turbidity not higher than 30% at day 7, all corn oil-in-water emulsions supplemented with 0.5% ERPI demonstrated a better stability than the emulsions prepared with olive oil.

Introduction

Corn and olive oil-in-water emulsions are major constituents of various foods, such as beverages, mayonnaise, salad dressings, and margarines (Depree and Savage, 2001; Mollakhalili Meybodi *et al.,* 2014). Corn and olive oils are among the most utilised edible plant oils in the food industry due to their valuable poly-unsaturated fatty acid profile, and relatively high thermal and storage stabilities (Konuskan *et al.,* 2019). In general, being comprised of two immiscible liquids, oil-in-water emulsions are thermodynamically unstable, and addition of emulsifiers is needed to achieve desired food products shelf life and organoleptic properties. As amphiphilic compounds, proteins are able to rearrange at oil/water interface and enhance repulsive steric forces among oil droplets, thus providing relatively high stability of the emulsions (Nikiforidis *et al.,* 2014; Shevkani *et al.,* 2015; Ghumman *et al.,* 2016).

Recent rapid world's population growth has driven the food industry toward more efficient and gentle utilisation of natural resources (Henchion *et al.,* 2017). Besides, customer's demand for healthier food, formulated with natural ingredients and additives, has increased. Being abundant and less expensive, plant proteins, including rapeseed/canola proteins, are considered an attractive alternative to animal proteins and synthetic compounds as functional ingredients (Wanasundara *et al.,* 2016).

Rapeseed meal is produced in high quantity as a by-product of vegetable oil extraction, reaching up to 48% of the rape seeds used (Ivanova, 2012). In recent

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years, the worldwide production of this by-product tends to increase due to the overall high demand at international markets for vegetable oils to be used either in food industry or for biodiesel generation (Zentková and Cvengrošová, 2013). Traditionally, rapeseed meal is utilised as a protein-rich ingredient in feed but only at a limited level due to the presence of anti-nutrients such as glucosinolates, polyphenols phytic acid, and high fibre content (Nega and Woldes, 2018). In recent years, preparation of protein isolates from industrial rapeseed meal, and their application in the food industry has been intensively studied in an attempt to find a better and more complete utilisation of this protein-rich source (Li and Guo, 2017). To prepare protein isolates for food purposes, a pre-treatment step of the rapeseed meal aiming at reducing the anti-nutrient level has been recommended (Purkayastha *et al.,* 2013; 2014). However, while the procedure is beneficial for anti-nutrients removal, modification of physico-chemical and functional properties of the protein isolates can be expected (Purkayastha *et al.,* 2014; Kalaydzhiev *et al.,* 2019a). Therefore, the properties of the protein-rich ingredients, obtained from pre-treated industrial rapeseed meal, needs to be studied and their applicability evaluated.

In a previous study, ethanol-treated rapeseed meal protein isolate (ERPI) was established as an efficient stabiliser in sunflower and rapeseed oil-in water emulsion (Kalaydzhiev *et al.,* 2019b). A dependence of the stability pattern of the sunflower and rapeseed oil-in-water emulsions on the type of the oil was established, and the need of a separate investigation when different oils are to be used in food formulation was identified. The aim of the present work was therefore to evaluate the potential of ERPI as an emulsifying agent in corn and olive oil-in-water emulsions under slightly acidic conditions (pH 6) at three protein and oil concentration levels. The emulsions initial stability was determined by evaluating the Gibbs free energy (ΔG) and particle size distribution, while the 7-day dynamics of the emulsion stabilities was investigated by turbidity measurements. The experimental design was intentionally chosen similar to a previous study (Kalaydzhiev *et al.,* 2019b) for a comparison reason. It was based on well-established methods and although simple, presented an efficient and relatively quick approach for the evaluation of the ERPI potential practical application.

Materials and methods

Materials

Industrial rapeseed meal was obtained from a local company and used for ethanol-treated rapeseed meal protein isolate (ERPI) preparation. The procedure is described in detail by Kalaydzhiev *et al.* (2019c). Briefly, the meal was grinded, sifted, and the particles sizing less than 0.315 mm were collected. Particles were then treated 4-times with 75% aqueous ethanol solution at a meal to solvent ratio of 25% (w/v) for 30 min at room temperature. The residue was dried and used for proteins alkaline extraction under optimised conditions, pH 12, 40°C, and continuous agitation for 60 min (Kalaydzhiev *et al.,* 2019d), followed by precipitation at pH 4.5 where the solubility of the protein was the lowest (Kalaydzhiev *et al.,* 2019c). The produced ERPI contained 86.86% of crude protein, as determined by Kjeldahl method (AOAC, 1990). The value 6.25 was used as a conversion factor in the calculation. Corn and olive oils of cooking grade used for the preparation of the emulsions, were bought from a local store. All the other reagents were of analytical grade.

Preparation of model food emulsions

A total of nine emulsions, combining three oil (5, 10, and 15% w/w) and three protein (0.25, 0.5, and 1.0% w/w) concentrations, as provided by ERPI, were generated for each type of oil (corn and olive) (Table 1). A 3^2 factorial design was used to assess the significant effect of two variables, oil and protein concentrations, on emulsions stability. All emulsions were prepared in a phosphate buffer (100 mM, pH 6) and constant homogenisation at 1,000 rpm (ISOLAB Laborgeräte GmbH, Germany) for 2 min. Sodium benzoate (0.5% w/w) and potassium sorbate (0.1% w/w) were added as preservatives to simulate food systems and minimise microbial development.

Evaluation of Gibbs free energy (ΔG, kJ mol $^{-1}$)

Gibbs free energy was determined as previously described (Kalaydzhiev *et al.,* 2019b), and calculated using Eq. 1:

$$\Delta G = - RT \ln K \quad \text{(Eq. 1)}$$

where, R = universal gas constant (R = 8.314 J/ (K. mol)), and T = absolute temperature (K). The equilibrium constant K was obtained by "dilution method" (Kendrow *et al.,* 2009). It was experimentally established as the slope of the absorbance at 350 nm (UV/Vis spectrophotometer, 1000E, Pharmacia Biotech Ultro-Spec, Cambridge, UK) as a function of emulsion serial dilutions with increasing bulk concentrations from 0.2 to 1.0% (v/v) with an increment of 0.2%.

Microstructural analysis

Microstructural characteristics of the

Table 1. Influence of oil and ERPI protein concentrations on Gibbs free energy (ΔG).

No. of sample	Oil type	Oil concentration (%)	ERPI protein concentration (%)	ΔG (kJ/mol)
1		5	0.25	-2.12 ± 0.03[a]
2		5	0.5	-3.60 ± 0.00[b]
3		5	1	-3.62 ± 0.02[b]
4		10	0.25	-3.76 ± 0.01[b]
5	Corn oil	10	0.5	-2.81 ± 0.42[a]
6		10	1	-3.36 ± 0.03[ab]
7		15	0.25	-4.51 ± 0.00[a]
8		15	0.5	-5.16 ± 0.06[b]
9		15	1	-5.08 ± 0.02[b]
1		5	0.25	-3.38 ± 0.01[b]
2		5	0.5	-2.64 ± 0.39[ab]
3		5	1	-2.30 ± 0.39[a]
4		10	0.25	-3.90 ± 0.02[c]
5	Olive oil	10	0.5	-3.57 ± 0.04[b]
6		10	1	-3.13 ± 0.04[a]
7		15	0.25	-4.66 ± 0.02[b]
8		15	0.5	-4.33 ± 0.02[a]
9		15	1	-4.31 ± 0.00[a]

Means within a column for a specific oil concentration with different lowercase letters differ significantly ($p < 0.05$).

emulsions were determined by using digital image processing. Emulsions microstructural profiles were determined within 5 min of preparation by following the same approach and equipment as previously described (Ivanova *et al.,* 2018). Images were conducted with a binocular microscope (BM-180 SP, Boeco, Hamburg, Germany) coupled with a digital video camera eyepiece (MDCE-5, Alltion Co., Ltd., Wuzhou, China). Presented particle size for each analysed sample was the average of the total number of measured objects which was in the range of 700 to 1,500.

Evaluation of emulsion stability by turbidity measurement

The emulsion stability was evaluated for a period of 7 d with 2-d intervals, and at a room temperature (23 ± 0.2°C). An aliquot of 0.2 mL from the bottom part of an emulsion was pipetted and diluted in a 100-mL volumetric flask with a phosphate buffer (100 mM, pH 6). The turbidity was measured at 350 nm against a control containing a phosphate buffer (pH 6), sodium benzoate, and potassium sorbate in concentrations used for the preparation of the model food emulsions (Ly *et al.,* 2008). The emulsion stability was evaluated by changes in sample turbidity relative to its initial value, expressed in percentage. Since unstable emulsions tend to form a relatively clear serum layer at the tube's bottom, the decrease in turbidity (%) at that part of the sample was used as an indicator of emulsion instability.

Statistical analysis

Statistical analysis was performed using Statistica© 6.0 (StatSoft, USA). Results were presented as means of at least three independent determinations ± standard deviation (SD). Mean differences were established by Fisher's least significant difference test for paired comparison at a significance level of $\alpha = 0.05$.

Results and discussion

Initial stability of emulsions

Initial stability of corn and olive oil-in-water emulsions was determined by two independent and significantly different methods, in an attempt to relate stability during storage at ambient temperature with

the corresponding initial characteristics. Emulsion stability is one of the most important characteristics, having a direct and significant impact on food product organoleptic properties and shelf life (Nikovska, 2012). To enhance the applicability of ERPI and the potential for technology transfer of data obtained, the present work was conducted with cooking grade corn and olive oils. The stability of the emulsions was evaluated at pH 6, which is typical for various food products, such as milk, meat, and some bakery products (McGlynn, 2003; Wanasundara *et al.,* 2016). As indicated by Gibbs free energy, the increase of oil concentration from 5 to 15% (w/w) increased the initial stability of corn- and olive oil-based emulsions (Table 1). The 15% corn oil emulsions, supplemented either with 0.5 or 1.0% ERPI protein, were characterised with the highest absolute negative values for ΔG (5.16 and 5.08 kJ/mol), meaning that they presented the highest initial stability. The Gibbs free energy is often used to measure spontaneity of a process (Mehta and Kaur, 2011). In the present work, the higher the negative value of ΔG, the more stable the emulsions were. Positive correlation between oil concentration and emulsion stability has been previously observed (Cortés-Muñoz *et al.,* 2009; Gandova and Balev, 2016; Hebishy *et al.,* 2017). By studying olive oil-in-water emulsions emulsified by soy protein or whey protein isolate, Nikovska (2012) also reported increased stability in response to the volume of oil phase increase, being associated with more closely packed droplets. Most probably, the enhancement of oil volume leads to increased hydrophobicity, which is a prerequisite for a better and more efficient adsorption of proteins (Mollakhalili Meybodi *et al.,* 2014; Hebishy *et al.,* 2017).

At each level of corn or olive oil concentration, levelling the ERPI protein concentration resulted in emulsions with significantly different stability, as evaluated by Gibbs free energy ($p < 0.05$) (Table 1). While in olive oil emulsions, the addition of ERPI protein in the lower range of the studied concentrations (0.25 or 0.5%) was sufficient to obtain the highest negative ΔG values (Figure 1B), a higher concentration of ERPI, either 0.5 or 1.0%, was needed to obtain corn oil-based emulsions with a better stability (Figure 1A). In the case of the emulsion containing 10% corn oil, 0.25% ERPI could be used since the result was not statistically different from the one obtained with 1.0% ERPI (Table 1).

The difference in the stability pattern of the emulsions, prepared with two different oils, might be due to the higher amount of the medium chain fatty acids (C_8 and C_{10}) contained in corn oil when compared with the olive oil (Kostik *et al.,* 2013). Taha *et al.* (2018) established that the emulsions with

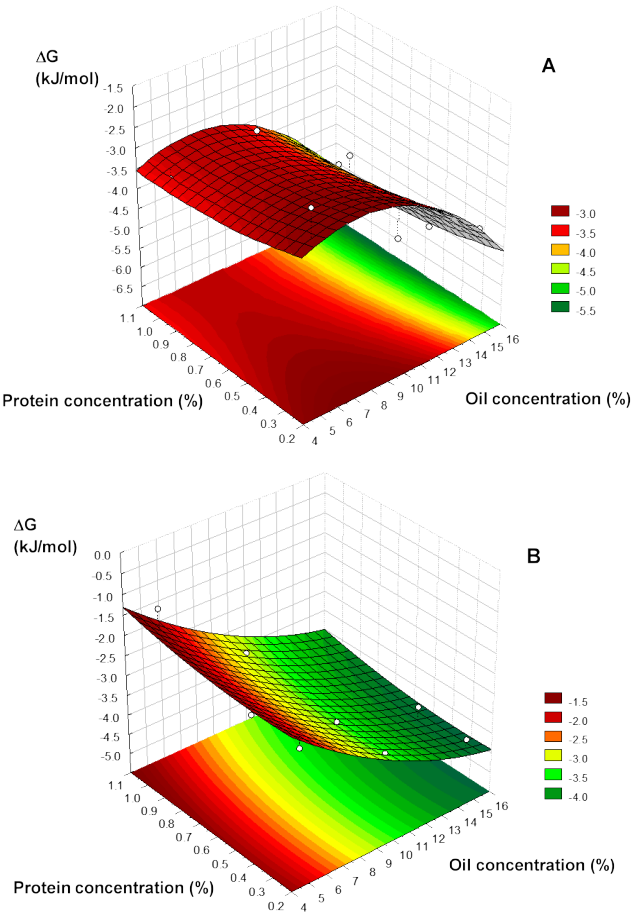

Figure 1. Combined effect of oil and protein (ERPI) concentrations on Gibbs free energy (ΔG) of corn oil-in-water (A), and olive oil-in-water (B) emulsions.

prevailing medium chain fatty acid content had higher adsorbed protein amounts at their interface than the emulsions containing high amounts of long chain fatty acids. Still, more experiments are needed to clarify interrelation between fatty acid profile of the oils and stability of the emulsions. In the present work, since at all corn oil concentrations, the supplementation of the emulsions with either 0.5 or 1.0% ERPI protein resulted in no significant differences on ΔG values (Table 1), from a practical reason, the lower concentration (0.5%) may be used for reducing emulsifier costs in food formulations.

Determination of emulsions microstructural characteristics is a different approach for stability evaluation (Molet-Rodríguez *et al.,* 2018). Emulsions with prevailing small-sized lipid particles and with homogenous distribution are expected to be relatively stable. This microstructural profile facilitates emulsifier/protein adsorption on lipid particles, thus reducing their coalescence and destabilisation (Hebishy *et al.,* 2017). In the present work, all samples were characterised with monomodal lipid particle size distribution, where droplets sizing 1 to

4.99 µm had the highest frequency except for the emulsion containing 15% olive oil and 0.25% ERPI (Figure 2 and 3). Microstructural profile of corn oil emulsions revealed that a 0.5% ERPI protein concentration (and also 1.0% for the 5% corn oil emulsion) is the most appropriate in the preparation of stable emulsions (Figure 2). This observation corresponds to ΔG values obtained where no statistical differences were observed for corn oil emulsions supplemented with 0.5 and 1.0% ERPI (Table 1). In contrast, for olive oil emulsion, there was no agreement on conclusions from the two methods. According to the microstructural analyses, for the olive oil emulsions, a 0.5% ERPI protein was needed to achieve higher stability (Figure 3), while 0.25% protein was required to achieve the highest stability as evaluated by Gibbs free energy (Table 1). Some discrepancies among microstructural analysis and ΔG value data have been observed by Kalaydzhiev *et al.* (2019b) when studying the stability of sunflower and rape seed oil-in-water emulsions. This observation was more pronounced in emulsions with higher oil volume and viscosity. According to Figueiredo *et al.* (2008), direct correspondence between Gibbs free energy and droplet morphology is highly dependent on physicochemical properties and concentrations of the oil and may not always be expected.

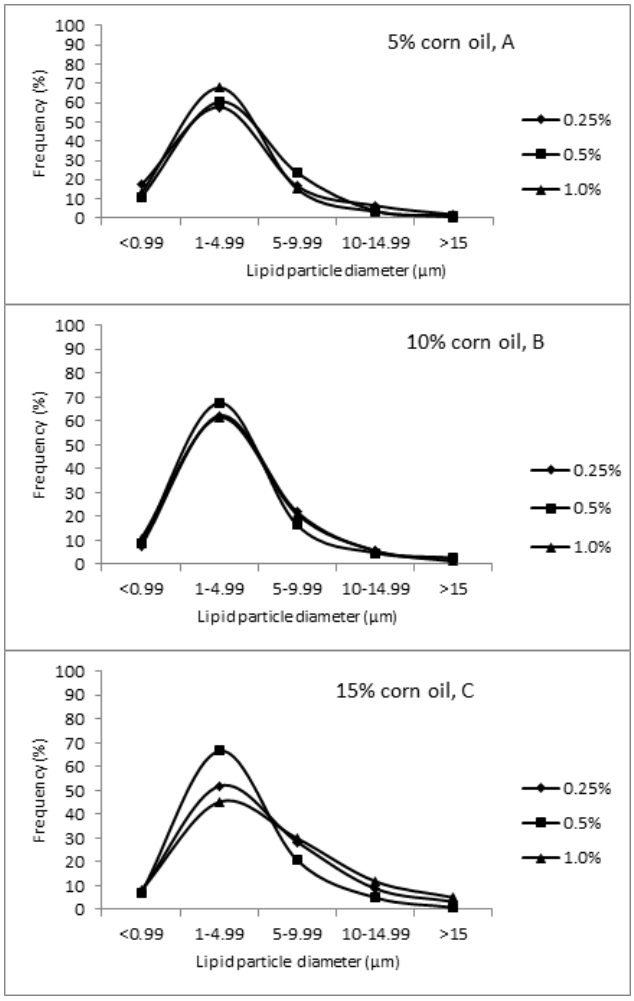

Figure 2. Lipid particle size distribution of emulsions prepared with 5% (A), 10% (B), and 15% (C) corn oil and different ERPI protein concentrations (0.25, 0.5, and 1.0%).

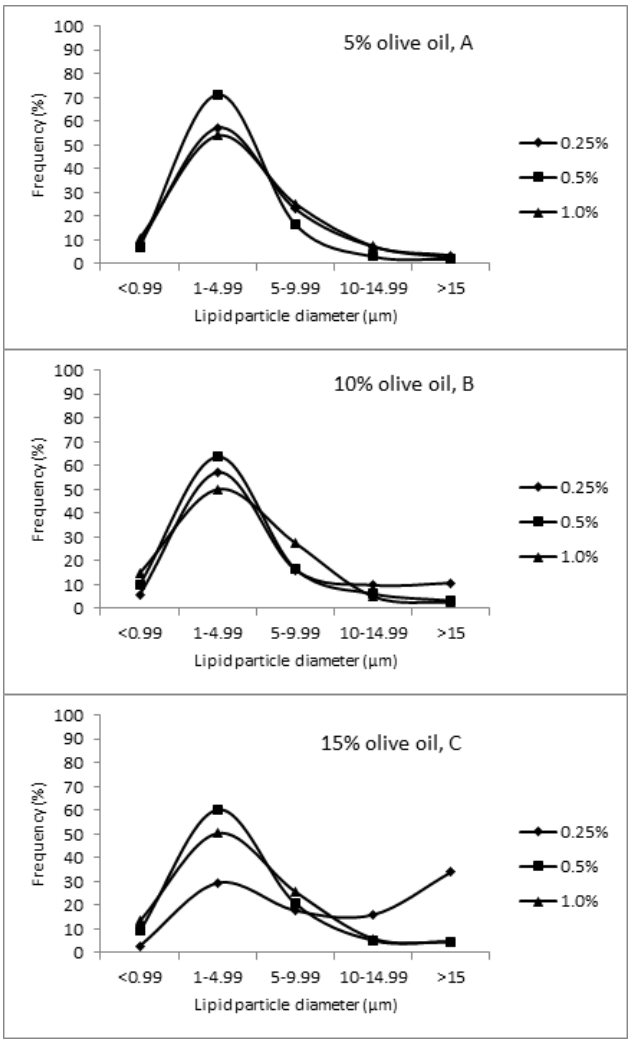

Figure 3. Lipid particle size distribution of emulsions prepared with 5% (A), 10% (B), and 15% (C) olive oil and different ERPI protein concentrations (0.25, 0.5, and 1.0%).

Dynamics of emulsion stability

Emulsions are structural parts of many natural or processed foods, and their shelf life stability is a significant factor, directly influencing sensorial properties and overall customers' perception (Serdaroğlu *et al.,* 2015). Following preparation, corn and olive oil-in-water emulsions were visually stable without phase separation. The 10 and 15% corn oil-in-water emulsions, supplemented with 0.5% ERPI protein (Figures 4B and 4C), demonstrated superior stability during the entire period studied, as compared to all the remaining samples (Figures 4

and 5). For these conditions, at day 7, the reduction of turbidity was lower than 30% (Figures 4B and 4C), exceeding the stability of sunflower and rapeseed oil-in-water emulsions previously investigated by Kalaydzhiev *et al.* (2019b). For comparison, when polysaccharides were used to stabilise beverage emulsions, a decrease of more than 70% in turbidity was observed at day 7 (Mikkonen *et al.,* 2009). In contrast to ΔG values (used to evaluate initial stability), where no significant differences between corn oil emulsions supplemented with 0.5 and 1.0% were observed (Table 1), those same samples exhibited distinguishable stability dynamics (Figures 4B and 4C). In contrast, the stability dynamics of olive oil emulsions showed compact behaviour regardless of different levels of ERPI supplementation (Figure 5). With a turbidity reduction lower than 52%, the results suggest that ERPI has a good potential as an emulsifier in food emulsions prepared with olive oil as well. In contrast to corn oil emulsions, the data generated on dynamics of the olive oil emulsion

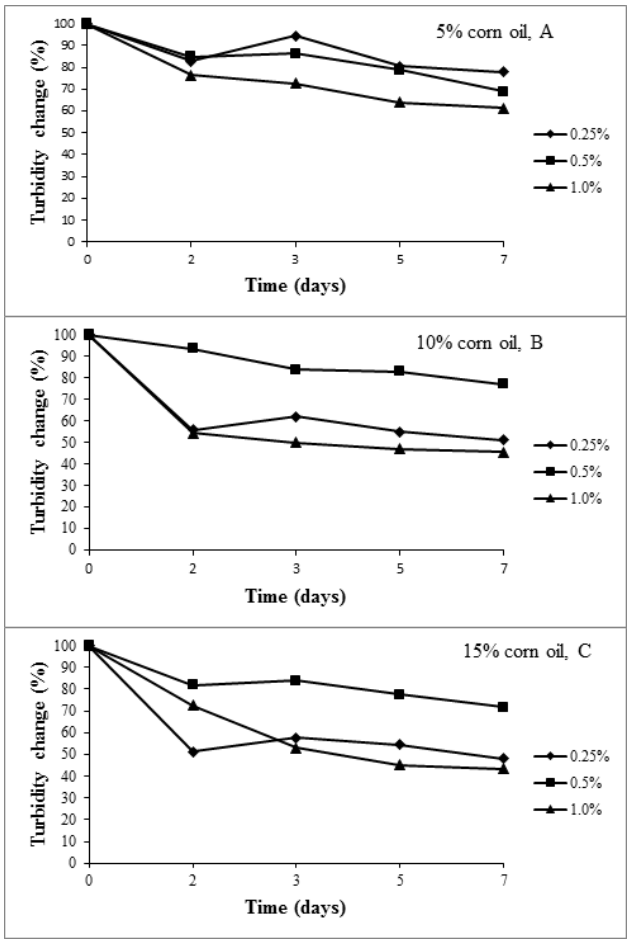

Figure 4. Stability dynamics of emulsions prepared with 5% (A), 10% (B), and 15% (C) corn oil and different ERPI protein concentrations (0.25, 0.5, and 1.0%). The means are from three independent measurements. Standard deviations do not exceed 0.51%.

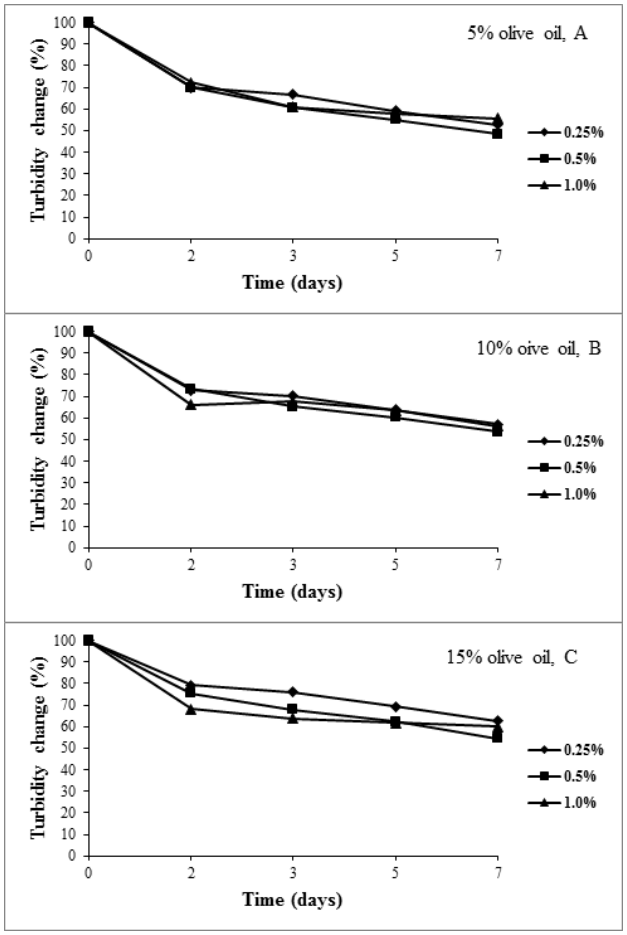

Figure 5. Stability dynamics of emulsions prepared with 5% (A), 10% (B), and 15% (C) olive oil and different ERPI protein concentrations (0.25, 0.5, and 1.0%). The means are from three independent measurements. Standard deviations do not exceed 0.48%

stability had a better correlation to Gibbs free energy values (Table 1), where the addition of 0.25% ERPI protein resulted in emulsions with the highest stability. In conclusion, a lack of correlation between initial stability and stability dynamics during storage may be expected, depending on types of the oil and protein concentrations used, and for practical reasons both evaluations are needed.

Overall, the corn oil-in-water emulsions exhibited better stability than olive oil-in-water emulsions. This might partially be attributed to different fatty acid profiles of the two oils. While not detectable in olive oil, caprylic (C_8) and capric (C_{10}) fatty acids are contained in relatively high amounts in corn oil, 4 and 7%, respectively (Kostik *et al.,* 2013). It has been established that medium-chain triglycerides, represented mainly by caprylic and capric fatty acids, have higher water solubility and lower interfacial tension as compared to long-chain triglycerides (Babayan, 1987). By studying the effect of different oils and ultrasound emulsification conditions on the physicochemical properties of emulsions stabilised

by soy protein isolate, Taha *et al.* (2018) observed that the emulsions containing medium-chain triglycerides were characterised with minimum droplet size and higher stability as compared to emulsions prepared with oils containing long-chain fatty acids. In addition, corn oil has lower viscosity than olive oil (Sahasrabudhe *et al.,* 2017). In general, it is considered that oils with lower viscosity are easier to break under external forces, and form smaller and uniformly distributed droplets (Molet-Rodríguez *et al.,* 2018). In a similar study, sunflower oil, which was characterised with a higher content of medium-chain fatty acids and lower viscosity than rapeseed oil, contributed to formation of emulsions with higher stability over a 7-d period (Kalaydzhiev *et al.,* 2019b).

Conclusions

The present work demonstrated the potential of ERPI as an emulsifying agent in corn and olive oil-in-water emulsions under slightly acidic conditions (pH 6). It is a novel ingredient which could serve as an alternative to emulsifying agents currently used in the food industry. The two types of emulsions exhibited different trends of stability, which were dependent on protein concentration and the type of the oil used. The oil composition and the specificities of the interaction between lipid molecules and proteins, used as emulsifiers, should be considered when generation of stable emulsions is needed. In both types of emulsions, the increase of oil concentration positively influenced their stability. Overall, the corn oil-in-water emulsions exhibited better stability than olive oil-in-water emulsions. These types of emulsions could be used for formulation of foods with a longer shelf-life.

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