



Design Optimization of emulsions based on fine fraction of durum wheat oil cake: Structural and rheological properties

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ABSTRACT

The production and stabilization of food-grade emulsions by using plant-derived particles have gained great attention due to the increasing demand for sustainable and plant-based alternatives. This study aims to explore the feasibility of using durum wheat oil and durum wheat oil cake, micronized and dry-fractionated (ff-DWOC), with no further chemical treatments, to produce emulsion gels. The emulsions obtained through a design of experiments (DoE) for mixture were analysed for their textural properties. The results of the textural parameters were subsequently modelled according to quadratic model. The nine emulsions were then characterized for their creaming index during 14 days of storage. The three most promising emulsions in terms of textural properties and creaming stability, were then characterized for their microstructure and rheological properties. The formulations with higher proportions of ff-DWOC and durum wheat oil affected the textural parameters, minimizing syneresis phenomena and serum phase separation, then resulting in excellent creaming stability over 14 days of storage. The emulsions containing 25% of ff-DWOC and 20 and 30% of oil displayed homogenous distribution of the oil droplets and a well-developed network, while emulsion with 15% ff-DWOC and 40% of oil (E7) showed a dense and compact continuous phase. According to the rheological results, the emulsions mentioned above exhibited a gel-like structures with $G' > G''$. E7 had the highest G' and G'' values, indicating a marked solid-like behaviour. These emulsions demonstrated compelling attributes, positioning them as strong contenders for incorporation into the food industry as innovative ingredients.

1. Introduction

Several commonly consumed foods are obtained through emulsification process, such as beverages, creams, desserts, butter and margarine, each with distinct characteristics in terms of appearance, texture, and flavour (Tan and McClements, 2021). Emulsions are thermodynamically unstable systems and can be kinetically stabilized by adding synthetic or natural emulsifiers (Verkempinck et al., 2018). In recent years, there has been a growing demand for plant-based foods, prompting researchers to find alternative and greener solutions for emulsion production and stabilization, avoiding the use of surfactants and animal-derived compounds (Calabrese et al., 2018; Drozłowska, Bartkowiak, & Łopusiewicz, 2020; Jafari, Doost, Nasrabadi, Boostani, & Van Der Meeren, 2020). Plant-derived particles, including polysaccharides (e.g. cellulose, starch, chitosan), proteins, fat crystals, phenolic, and phytosterol particles have shown promising properties as

a stabiliser for food-grade emulsions (Jafari et al., 2020; Luo et al., 2012; Zafeiri, Smith, Norton, & Spyropoulos, 2017). Moreover, using plant-based alternatives has various advantages, such as a lower risk of contamination, alignment with cultural eating habits, and economic benefits over their animal counterparts (Hossain, Deeming, & Edler, 2021; Hu et al., 2021; Jafari et al., 2020). The molecules aforementioned can be extracted from fruits, vegetables, pulses, cereals, and/or their waste and by-products whose management and valorisation have always represented a critical point to ensuring major sustainability of food industry (Droźłowska et al., 2020; Hu et al., 2021; Low, Siva, Ho, Chan, & Tey, 2020; Martínez-Martí, Quiles, Moraga, Llorca, & Hernando, 2022). For instance, oat bran extract obtained from food by-product steams has been used as a natural emulsifier, producing emulsions resistant to a wide range of pH, heat, and time-induced stresses (Ralla et al., 2018). Similarly, the aqueous microfibrillated cellulose (MFC) suspension prepared by the extraction of ground mangosteen (*Garcinia mangostana*

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L.) rind revealed good physical properties, allowing to obtain an emulsion with high stability (Winuprasith & Suphantharika, 2013). However, the selective use of these molecules and their functionalization, requires extraction and purification processes, as well as chemical treatments, which involves a high energy and economic expenditure, in addition to have a negative impact on the environment (Rawal, Annamalai, Bhandari, & Prakash, 2023).

Thus, the actual challenge lies not only in reusing waste and by-products, but also in reducing the treatments to which they are subjected, focusing more on the intrinsic characteristics of the raw materials, especially if they are considered biocompatible, sustainable and their reuse in line with the clean-label approach (Boostani, Hosseini, Marefati, Hadi, & Rayner, 2020; López-García, Moraga, Hernando, & Quiles, 2021; Martínez-Martí et al., 2022; Zhu, 2019). In light of this, Huc-Mathis et al., 2020 investigated about the potential emulsifying properties of two by-products, oat bran and apple pomace, only dried and micronized, without additional processes and/or the use of solvents to separate and purify the desired molecules. Both powder-stabilized emulsion showed gel-like behaviours over a two weeks period, with no creaming separation. Recently, Martínez-Martí et al. (2022) tested citrus pomace (CP), which displayed favourable stabilizing and emulsifying properties. This enabled the creation of stable high internal phase emulsions (HIPes) with a solid-like behaviour. Additionally, Sridharan, Meinders, Bitter, and Nikiforidis (2020) highlighted no significant differences in terms of physical features between emulsions obtained with pea flour and pea protein concentrate. Furthermore, as a result of their structural and mechanical properties, emulsion systems have found several applications in the food sector as carriers of bioactive compounds or for the production of innovative foods with improved nutritional and textural characteristics (Akçiçek et al., 2022; Muñoz-González, Ruiz-Capillas, Salvador, & Herrero, 2021; Zeng et al., 2017). A number of studies have used emulsions as fat-replacers in bakery (Giarnetti, Paradiso, Caponio, Summo, & Pasqualone, 2015; Milićević et al., 2020; Xie et al., 2021), meat (De Souza Paglarini et al., 2021; Serdaroglu, Öztürk, & Uргу, 2016; Utama, Jeong, Kim, & Lee, 2018) and dairy products (Anvari & Joyner, 2019; Li et al., 2022; Zhao, Khalesi, He, & Fang, 2023). Moreover, emulsion gels can be utilized as basic ingredient for spreadable plant-based cheese (Mefleh et al., 2022), salad dressing (Akçiçek and Karasu, 2018; De Angelis, Squeo, Pasqualone, & Summo, 2022; Lu et al., 2021) or low fat mayonnaise (Akciçek et al., 2022; Chivero, Gohtani, Yoshii, & Nakamura, 2016). Durum wheat supply chain results particularly interesting considering the large amount of by-products (e.g. bran, germ, and various middlings) produced during the milling process, which should be upcycled following circular economy principles (Vurro et al., 2022). These by-products have proven to be suitable for oil extraction, which appears to be a rich source of polyunsaturated fatty acids (mainly linoleic), tocopherols, tocotrienols, carotenoids, phytosterols, and policosanol, making it nutritionally attractive as an alternative to other oils (Squeo et al., 2022). In addition, oil extraction yields another valuable by-product known as defatted durum wheat oil cake. Through a micronization and dry-fractionation process, this by-product can be transformed into two distinct fractions: a fine fraction, abundant in protein, and a coarse fraction, enriched with starch (Squeo et al., 2023). These fractions can be effectively utilized as novel ingredients for enhancing the nutritional profile of food (Squeo et al., 2023). According to literature through micronization and dry-fractionation process could enhance the technological functionality of flours (Hémery et al., 2010; Martín-Esparza et al., 2018). Dry-fractionation technique allows for the preservation of the protein native state, minimizing structural modifications and offering superior functional properties (e.g. proteins solubility, emulsions stability or gelling properties) in food applications (Ciccoritti, Nocente, Sgrulletta, & Gazza, 2019; De Angelis et al., 2020; Silventoinen, Rommi, Holopainen-Mantila, Poutanen, & Nordlund, 2019). Moreover, dry-fractionation emerges as a sustainable and efficient method, producing concentrates enriched in valuable components with lower energy

consumption (Silventoinen et al., 2019). In this context, the aim of the present study was to explore the feasibility of using durum wheat oil and the fine fraction of durum wheat oil cake, also by applying the Design of Experiments, to produce stable emulsions by evaluating the influence of the ingredients on the structural and rheological properties.

2. Material and methods

2.1. Raw materials

Durum wheat oil and oil-less and fine fraction of durum wheat oil cake were kindly provided by Casillo Next Gen Food Srl (Corato, Puglia, Italy). Oil and hexane residues were removed from durum wheat oil cake, then the by-product was subjected to micronization and dry-fractionation process, obtaining a fine fraction of durum wheat oil cake (ff-DWOC) (Squeo et al., 2023). Specifically, as described in the mentioned study, the micronized durum wheat oil cake was subjected to an air classification process, which allowed to separate, based on the particles size, a fine fraction rich in proteins from a coarse one rich in starch. Therefore, ff-DWOC consisted of proteins (30.7%), dietary fibres (26.5%), carbohydrates (23.9%), ashes (10.9%), moisture (7.5%) and fats (0.5%).

2.1.1. Physico-chemical and functional properties of fine fraction of durum wheat oil cake

Bulk density (BD), water absorption capacity (WAC), and oil absorption capacity (OAC) were assessed according to Summo et al. (2019) with some modifications. The bulk density is an important parameter, which refers to the behaviour of a product in a dry mixture and varies with the finesses of particles, influencing the texture of foods (Chandi & Sogi, 2007; Summo et al., 2019). For OAC, 0.75 g of ff-DWOC were mixed with 9 mL of durum wheat oil in pre-weighed centrifuge tubes. The mixtures were stirred for 1 min and after 30 min, the tubes were centrifugated at 3000×g for 20 min. The supernatant was separated and the excess of oil was removed by tilting and draining the tubes for 25 min. WAC and OAC was expressed as gram of water/oil bound per gram of powder. Each measurement was carried out in triplicate.

2.2. Experimental domain and D-optimal design settings

The design of experiments (DoE) for mixture was applied to better understand the ingredients influence on the emulsions textural properties. The experimental domain was defined according to the following ranges of the ingredients and expressed as g 100 g⁻¹: ff-DWOC (5 ≤ X1 ≤ 25), water (45 ≤ X2 ≤ 65), durum wheat oil (10 ≤ X3 ≤ 50). Once defined the experimental domain, the experimental points were chosen by means of D-optimality criterion, according to the quadratic model: $Y = \beta_1x_1 + \beta_2x_2 + \beta_3x_3 + \beta_{12} \times 1 \times 2 + \beta_{13} \times 1 \times 3 + \beta_{23} \times 2 \times 3 + \epsilon$, where Y, x and b are the response variables, the components under study, and the model coefficients, respectively. Table 1 reports the nine experiments (E1-E9) chosen from all the possible experiments in the experimental domain.

Table 1

Emulsion formulation according to the D-Optimal mixture design (g 100 g⁻¹). ff-DWOC = fine fraction of durum wheat oil cake.

Formulation	ff-DWOC (X1)	Water (X2)	Durum wheat oil (X3)
E1	25	65	10
E2	25	55	20
E3	15	65	20
E4	25	45	30
E5	15	55	30
E6	5	65	30
E7	15	45	40
E8	5	55	40
E9	5	45	50

2.3. Emulsions preparation

The emulsions were prepared according to López-García et al. (2021) with slight modifications. The ff-DWOC was dispersed in water and homogenized using an Ultra-Turrax T-18 (IKA-Werke GmbH & Co. KG, Staufen, Germany) at 8000 rpm for 4 min. Next, durum wheat oil was gradually added, homogenizing at 14,000 rpm for 4 min. Finally, the emulsion was homogenized at 16,000 rpm for 30 s. The resulting emulsions were stored at 4 °C overnight and analysed at 25 °C.

2.4. Characterisation of emulsions

2.4.1. Back extrusion

The back extrusion was carried out using a TA.XT. plus Texture Analyser (Stable, Microsystem, Godalming, UK) equipped with an A/BE-D40 back extrusion cell (40 mm diameter) using a 30 kg load cell. The samples (50 g) were placed into an extrusion cylinder (50 mm internal diameter and 75 mm height). The analysis consisted in a single compression cycle at 1 mm s⁻¹ and a distance of 15 mm. The following parameters were measured: *Firmness* (g), the maximum force recorded during the compression; *Consistency* (g.s), the area recorded during the compression; *Cohesiveness* (g), which represents the maximum negative force recorded during the compression, thereby, the more negative is the value, the higher is the cohesiveness of the emulsion; *Index of viscosity* (g.s), which is the negative area of the graph and represents the resistance of the sample to flow off during the probe returns to the starting position. Each measurement was performed in triplicate.

2.4.2. Creaming index

The creaming index (CI) was evaluated according to the method reported by Keowmaneechai and McClements, 2002. Ten grams of each emulsion were placed in a glass test tube, sealed with plastic caps to prevent evaporation and kept at 4 °C. The total height of the emulsion and the cream layer were measured with a digital calliper after 1, 7, and 14 days. The CI was calculated using the following equation:

$$\text{Creaming index (\%)} = (H_s / H_t) \times 100 \quad (1)$$

Where H_t is the total height of the emulsion (mm) and H_s is the height of cream layer (mm). Each measurement was performed in triplicate.

2.4.3. Microstructure analysis

2.4.3.1. Light microscope. The emulsions were observed through a light field microscope (LM) (Leica DM5000, Leica, Wetzlar, Germany), using a × 20 objectives lens at room temperature. The software ImageJ was used to determine the diameter of the oil droplets, taking an average of 100 droplets.

2.4.3.2. Confocal laser scanning microscopy (CLSM). Confocal laser scanning microscopy (CLSM) analysis was carried out using a ZEISS LSM 780 Axio Observer.Z1 laser scanning microscope (Carl Zeiss, Germany) with a C-Apochromat 40X/1.20 W corrective water immersion objective lens. Rhodamine B (Fluka, Sigma-Aldrich, Missouri, USA) solubilized in distilled water 0.2% was used to stain proteins and carbohydrates, while Nile Red (Fluka, Sigma-Aldrich, Missouri, USA) was solubilized in PEG 200 at 0.1 g L⁻¹ and used to stain oil. The detection wavelength were 570 nm and 515 nm for Rhodamine B and Nile Red, respectively. To observe and study the emulsions, a drop of sample was placed on a slide, 20 µL of Nile Red solution was added and it was left to rest for 10 min. The same procedure was performed with Rhodamine B, and samples were covered with a glass coverslip. The images were taken and stored at 1024 × 1024 pixels resolution using the microscope software (ZEN).

2.4.3.3. Cryo-field emission scanning electron microscopy (Cryo-FESEM). The Cryo-FESEM analysis was carried out using an Ultra55 FESEM

microscope (Zeiss, Oberkochen, Germany). The samples were immersed in slush nitrogen and then fractured, etched, coated with platinum, and observed at 15 kV at a working distance of 15 mm.

2.4.4. Rheological analysis

The rheological properties of the emulsions were measured using a rheometer HAAKE MARS iQ Air (Thermo Fisher Scientific Inc., Waltham Massachusetts, USA). The emulsions were analysed at a temperature of 25 °C using a plate-plate geometry and a gap height of 1.0 mm. The measurements were carried out within the linear visco-elastic region identified through the *amplitude sweep test* (frequency of 1 Hz and deformation in a range of 0.05 and 100%). The *frequency sweep test* was carried out in the frequency range from 0.1 to 10 Hz, at constant amplitude of 0.2% strain and temperature of 25 °C. The measurements were performed in triplicate.

2.5. Statistical analysis

The mixture experimental design was set up and analysed using CAT (Chemometric Agile Tool) software, freely downloadable from <http://gruppochemiometria.it/index.php/software>, accessed on November 5, 2023. The experimental data were expressed as the mean ± standard deviation (n = 3) and subjected to one-way and two-way ANOVA, followed by a Tukey's HSD test. Significant differences among values of all the parameters were determined at *p-value* < 0.05 by Minitab 17 Statistical Software (Minitab, Inc., State College, PA, USA, 2010).

3. Results and discussion

3.1. Functional properties of ff-DWOC

The techno-functional properties of ff-DWOC were evaluated considering the bulk density (BD), water absorption capacity (WAC), and oil absorption capacity (OAC) (Table 2). As regards, the ff-DWOC showed a bulk density of 0.62 g mL⁻¹, which is in line with the values of durum wheat flour found by Siddiq, Nasir, Ravi, Dolan, and Butt (2009), but significantly higher than rice bran protein concentrates, which ranged from 0.12 to 0.21 g mL⁻¹. The water and oil absorption capacity refer to the amount of water and oil retained by 1 g of powder. The WAC can be attributed to the presence of those hydrophilic molecules like dietary fibre, proteins in particular polar amino acids residues that have great affinity for water molecules (Awuchi et al., 2019; De Angelis et al., 2020; do Carmo et al., 2020; Lian, Luo, Gong, Shi-Ji, & Serventi, 2019). The ff-DWOC showed a WAC of 1.37 g g⁻¹, consistent with WAC values reported for legume flours found by Du, Jiang, Yu, and Jane (2014) ranging of 1.12 g g⁻¹ to 1.89 g g⁻¹, and significantly higher than the values found by Bashir, Swer, Prakash, and Aggarwal (2017) on the whole wheat flour before and after different doses of gamma irradiation. However, Chandi and Sogi (2007) highlighted considerably higher WAC values for rice bran protein concentrates, which could be attributed to the elevated nitrogen solubility identified. Moreover, proteins play a key role in OAC, thus proteins with higher hydrophobicity exhibit enhanced lipid binding, due to the ability of non-polar side chain of amino acids to form hydrophobic interaction with hydrocarbon chains of lipids (Du et al., 2014; Godswill, 2019). In terms of OAC, it was found a value equal to 1.51 g g⁻¹, which was notably higher when

Table 2
Functional properties of ff-DWOC.

BD (g mL ⁻¹)	0.62 ± 0.01
WAC (g g ⁻¹)	1.37 ± 0.09
OAC (g g ⁻¹)	1.51 ± 0.02

BD, bulk density; WAC, water absorption capacity, OAC, oil absorption capacity. The values represent mean of triplicates ± standard deviation.

compared to the values obtained for pulses and wheat flours, as analysed by Ferawati, Hefni, and Witthöft (2019). Nevertheless, the OAC value of ff-DWOC resulted lower than that of chickpea protein treated with enzymatic cross-linking combined with ultrasound (Zhu et al., 2022). Zhu et al. (2022) reported that ultrasonic treatment and enzymatic cross-linking enhanced the OAC by improving the surface hydrophobicity and modifying the proteins structure, thereby increasing the ability to capture more fat and consequently the oil absorption capacity of the protein.

3.2. Emulsions characterisation

3.2.1. DoE approach for analysing textural properties of the emulsions

Textural properties of emulsions are considered critical for their processing characteristics and application fields (Liu et al., 2020). Obtaining an emulsion with the right level of firmness and stability poses a significant challenge for researchers (Patel, Nicholson, & Marangoni, 2020). Fig. 1 depicts the contour plots of firmness (g), consistency (g-s), cohesiveness (g), and index of viscosity (g-s) of the nine emulsions. The DoE is a procedure that provides a comprehensive exploration of how different components interact within a product, by studying the effect resulting from proportional variations of ingredients rather than considering their absolute variation. Therefore, this approach allowed us to better understand how the three ingredients (factors) – ff-DWOC (X1), water (X2) and durum wheat oil (X3) –

influenced the textural properties of emulsions and to identify the best formulations in terms of texture and physical stability for future applications. The fitting of the responses for all the back extrusion parameters had a $R^2 \geq 0.90$, which is considered an excellent value (Table 3) (De Angelis et al., 2021). Moreover, the variables displayed an almost perfect linear correlation (~ 1.00) and for this reason, analysing the response for one parameter it would be possible to estimate the response of the other parameters. The textural properties are the result of several interactions and the formation of complex between protein, carbohydrates, starch and lipids (Bortnowska, Balejko, Tokarczyk, Romanowska-Osuch, & Krzemińska, 2014). Considering the contour plots, it was observed that moving towards X1 (ff-DWOC) and X3 (durum wheat oil) vertices, firmness, consistency (blue zone), viscosity

Table 3

Regression coefficients of the model for all the responses measured on the emulsions produced by the DoE.

Quadratic model	R ²	Q ²	SD res	RMSECV
Firmness	0.90	0.87	39.48	44.46
Consistency	0.93	0.91	489.6	551.7
Cohesiveness	0.92	0.90	53.9	60.74
Index of viscosity	0.94	0.92	64.35	72.86

Abbreviations: R², adjusted coefficient of determination; Q², coefficient of determination in cross-validation; SD res, standard deviation of residual, RMSECV, root mean error in cross-validation.

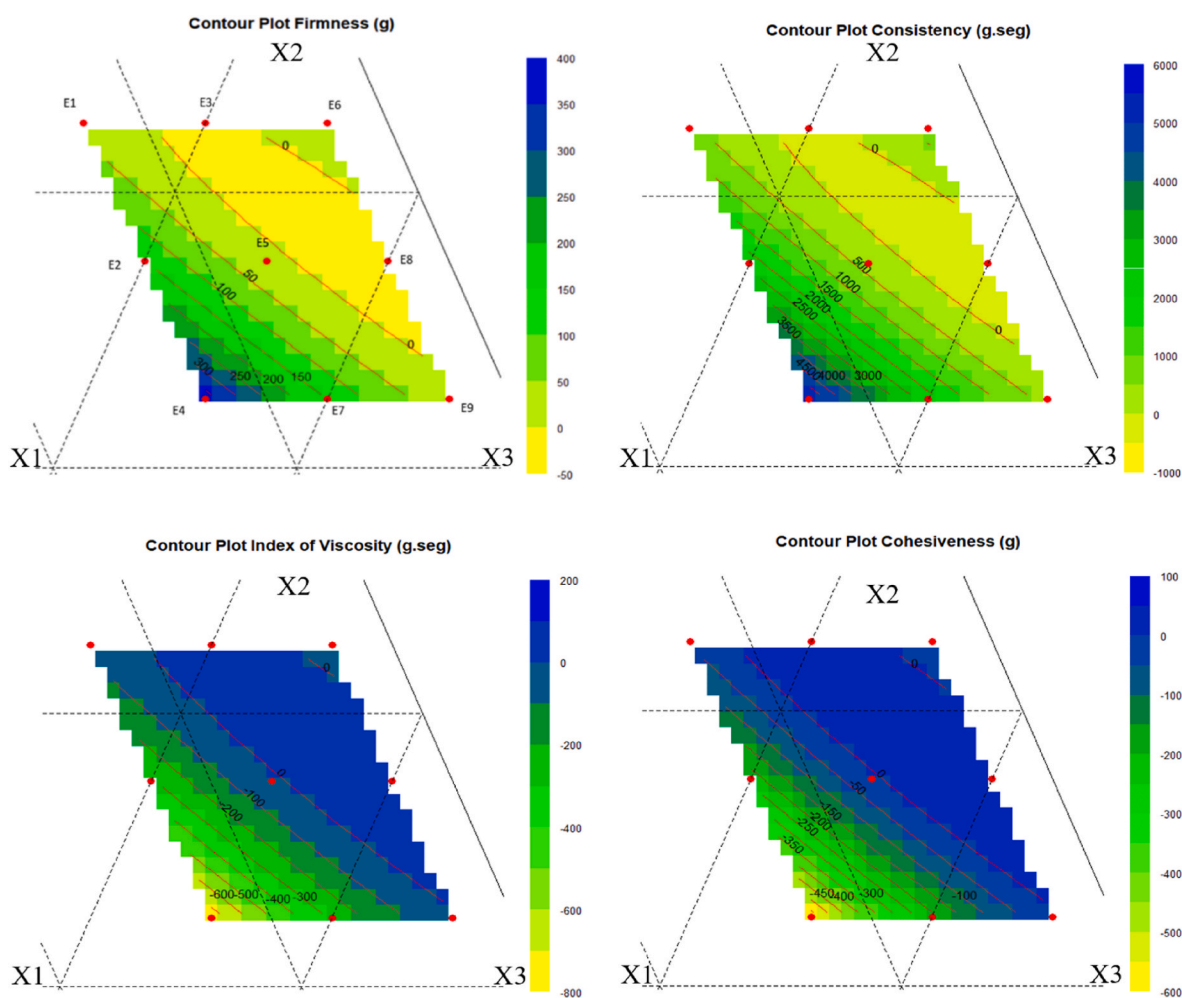


Fig. 1. Contour plots from D-optimal design of experiments (DoE) of firmness (g), consistency (g-s), index of viscosity (g-s) and cohesiveness (g) of the experimental emulsion obtained by D-optimal DoE. X1 = ff-DWOC; X2 = water; X3 = durum wheat oil. Red points represent the nine emulsions chosen within all the experimental domain.

Table 4

Textural parameters of the emulsions (E1-E9) evaluated by back extrusion of the experiments carried out according to the D-optimal mixture design.

Sample	Firmness (g)	Consistency (g·s)	Cohesiveness (g)	Index of viscosity (g·s)
E1	44.660 ± 3.187c	726.527 ± 28.790c	-51.354 ± 6.054 b	-86.347 ± 2.681 b
E2	96.870 ± 5.598 b	1668.919 ± 52.875 b	-162.180 ± 8.555c	-230.737 ± 11.715c
E3	19.976 ± 0.463cd	319.693 ± 7.245 d	-13.757 ± 1.443a	-6.724 ± 2.271a
E4	420.271 ± 24.183a	6072.564 ± 279.468a	-639.019 ± 32.795 d	-835.603 ± 65.733 d
E5	32.781 ± 2.010cd	476.574 ± 59.091cd	-28.946 ± 2.700 ab	-38.504 ± 9.477 ab
E6	14.677 ± 2.206 d	275.776 ± 47.665 d	-8.407 ± 2.672a	-2.264 ± 1.142a
E7	106.661 ± 8.717 b	1724.367 ± 96.542 b	-153.732 ± 4.424c	-226.246 ± 9.817c
E8	18.599 ± 2.675 d	309.289 ± 57.798 d	-7.038 ± 1.986a	-0.313 ± 0.151a
E9	19.175 ± 1.769cd	311.697 ± 18.108 d	-14.781 ± 1.423a	-12.133 ± 2.321a

Different letters in the same column mean significant differences (p-value <0.05) according to one-way ANOVA and the Tukey's HSD test.

and cohesiveness increased (yellow zone). Regarding the contour plots of firmness, it can be observed that some samples appear to fall within the yellow zone, indicating values below 0. However, considering the error associated with the model (represented by SD and RMSECV), such results are attributed to the uncertainty in estimating the value and do not hold physical significance. E4 with the combination of ff-DWOC (25%) and durum wheat oil (30%) at the greatest levels, displayed the highest value of firmness (420.271 ± 24.183 g), followed by E2 (25% ff-DWOC and 30% of durum wheat oil) and E7 (15% of ff-DWOC and 40% of durum wheat oil), which did not show statistically significant differences. Therefore, the presence of dietary fibres that can interact with water and oil may have promoted the formation of entangling network, increasing the viscosity of the emulsion (Román, Martínez, & Gómez, 2015; Rudra et al., 2020). In line with our results, Song, Zheng, Ma, Kang, and Ren (2020) observed an increase of gel strength with an increasing concentration of solid particles. In addition to solid particles, oil also plays a fundamental role in influencing the structural characteristics, as observed by Ma et al. (2013), where an increase in amount of oil resulted in an increment in the firmness and viscosity of the salad dressing. On the contrary, moving towards X2 (water) vertex, it was observed a decline of the textural parameters (see samples E1, E3 and E6) as found by De Angelis et al. (2021). Therefore, by reducing amount of ff-DWOC and durum wheat oil a low viscosity index and consequently a less firm and cohesive structure were observed (see Table 4).

3.2.2. Physical stability-creaming index

Creaming index (CI) analysis provides information about the aggregation level of particles constituting the emulsion and its stability. It is known that particles with a higher density tend to settle, while those with lower values rise up forming a creamy layer (Wang, Li, Wang, & Özkan, 2010c). Table 5 shows the trend of CI of emulsions during a storage period of 14 days. According to the two-way ANOVA, both formulation and time of storage influence the physical stability of emulsions having a p-value < 0.01. Specifically, in the emulsions characterized by a content of solid particles of 5%, E6, E8, and E9, the separation of the two phases occurred after 24 h of storage. After 7 days of storage, the separation occurred in E3 and E5 containing 15% of solid particles and, respectively, 20 and 30% of oil; however, E5 displayed a significantly lower value of CI than E3. Regarding E1, it exhibited good physical stability until 7 days of storage, but the 14th days of storage the CI reached a value equal to 22.27% that could be related to the greater amount of water (65%), which induced an increase of coalescence rates

Table 5

The creaming index (CI) of the emulsions (E1-E9) at different storage days (1, 7, and 14 day).

Samples	Days of storage		
	1	7	14
E1	0.00 ± 0 h	0.00 ± 0 h	22.27 ± 1.38 c
E2	0.00 ± 0 h	0.00 ± 0 h	0.00 ± 0 h
E3	0.00 ± 0 h	13.57 ± 2.04 f	15.14 ± 1.12 def
E4	0.00 ± 0 h	0.00 ± 0 h	0.00 ± 0 h
E5	0.00 ± 0 h	4.31 ± 0.49 g	4.49 ± 0.06 g
E6	15.75 ± 0.31 de	29.31 ± 1.35 b	33.28 ± 1.25 a
E7	0.00 ± 0 h	0.00 ± 0 h	0.00 ± 0 h
E8	14.78 ± 0.52 ef	16.39 ± 0.82 de	17.23 ± 0.46 d
E9	5.44 ± 0.08 g	5.66 ± 0.25 g	5.82 ± 0.05 g

Different letters mean significant statistical differences (p-value <0.05) according to two-way ANOVA (factors: formulation*time of storage).

and destabilization kinetics (Ghasemi, Darjani, Mazloomi, & Mozaffari, 2020). Otherwise, E2, E4, and E7 showed a good physical stability during all the storage period. Therefore, the combination of higher amount of oils and solid particles with higher viscosity of continuous phase could reduce the mobility of the oil droplets and their lower upward movement according to Stokes' law, increasing the resistance to separation (McClements & Li, 2010; Wang et al., 2010c; Hosseini, Jafari, Mirzaei, Asghari, & Akhavan, 2015; Winuprasith et al., 2013; Drozłowska et al., 2020; Rudra et al., 2020). Moreover, the hydrodynamic interaction among oil droplets determines a decrease of creaming rates, thus concentrated emulsions exhibit a superior resistance to creaming compared to their dilute counterparts (Dickinson, 2009, pp. 23–49). Remarkably, the high oil concentration determined a densely packed emulsion droplets, modifying not only the textural behaviour but also the stability of the system during storage, limiting the syneresis phenomena (Ma et al., 2013; Chivero et al., 2016). This phenomenon underlines the importance of achieving high oil volume to enhance stability against creaming. Rawal, Annamalai, Bhandari, and Bhandari (2023) and Valoppi, Wang, Alt, Peltonen, and Mikkonen (2021) observed a reduction of serum phase separation and a significantly decrease of CI when the oil and solid particles content increase. Specifically, Rawal et al. (2023) highlighted an even better CI (0.0%) in the emulsion prepared with mild chemically treated oat particles and 40% of oil (CH40) compared to that with 10% of oil (CH10) and its control counterpart (CO40). On the other hand, Valoppi et al. (2021) linked better creaming stability of emulsions to an increase of soluble proteins and volume fraction of fibres added coupled with the homogenization process. In light of the results obtained, it was decided to proceed with microstructural and rheological properties the three samples (namely E2, E4, and E7) that showed the highest values of the considered textural parameters and the best creaming stability.

3.2.3. Microstructure analysis

Among the various methods developed for the morphological evaluation of emulsions, microscopy ones appear to be the most rapid and simple, providing information about morphology, droplet size, flocculation, and coalescence phenomena (Low et al., 2020; McClements and Gumus, 2016). Fig. 2 shows the LM, CLSM and Cryo-FESEM images of the three emulsions chosen from the results obtain in terms of textural properties and stability. The three emulsions chosen, E2, E4, and E7, showed a fine and compact structure, with the oil droplets highly packed in the system (Fig. 2, LM images: A, B and C), which correlates with the favourable values obtained for creaming index and back-extrusion parameters. Specifically, E4 had the smallest droplets with a diameter of 3.41 ± 0.35 µm, confirming the result obtained in terms of firmness, in fact according to Olsson, Håkansson, Purhagen, and Wendin (2018), the decrease in particles diameter leads to a firmer structure of the emulsion. E2, which differed from E4 by a lower oil content (20%), was characterised by a slight increase of particle diameter 4.39 ± 0.31 µm. As

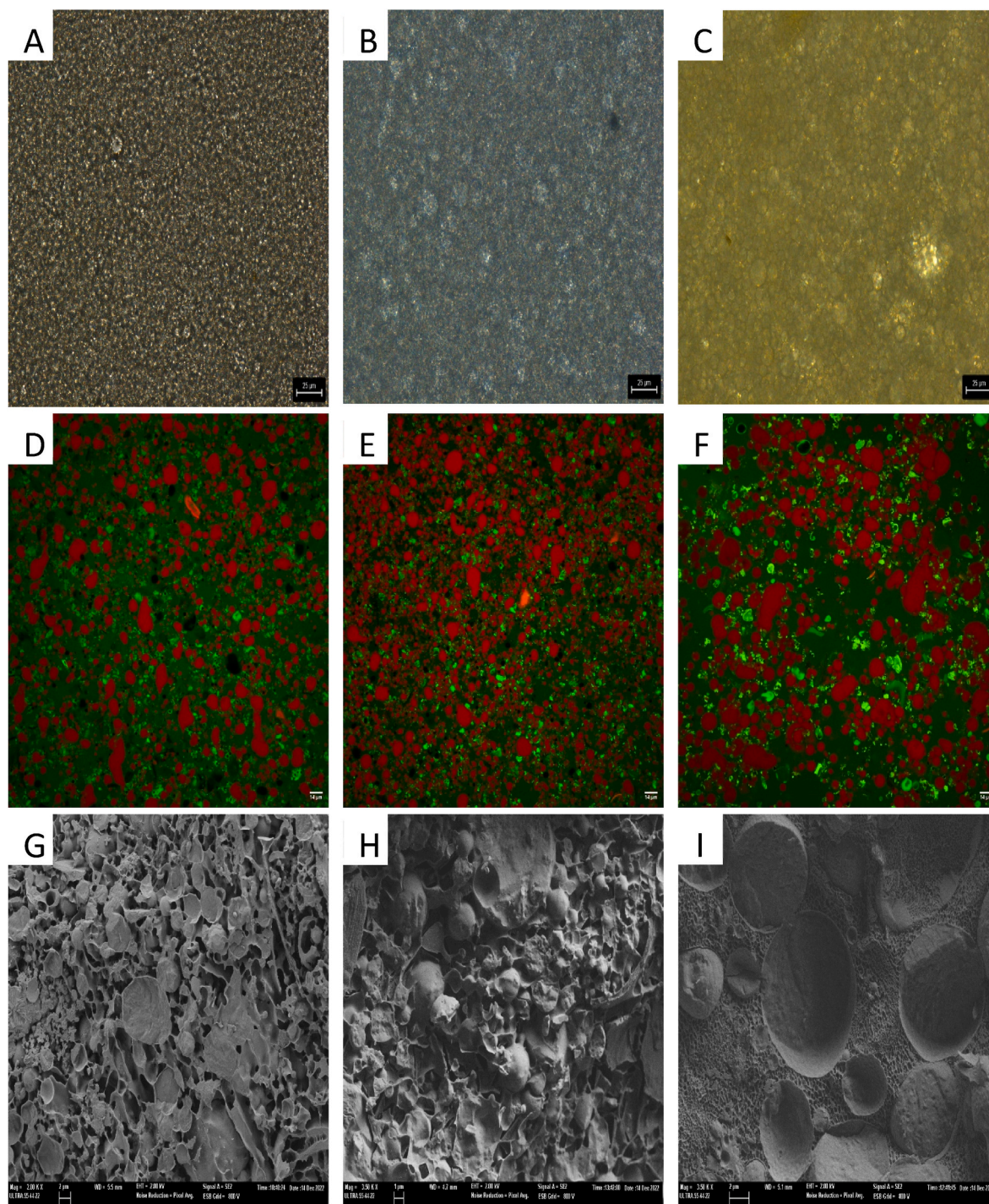


Fig. 2. Microstructural images of emulsions E2, E4 and E7. First row: LM images, scale bar = 25 µm; second row: CLSM images, scale bar = 14 µm; third row: Cryo-FESEM images, scale bar = 2 µm. A,D,G: emulsion E2. B,E, H; emulsion E4; C, F, I: emulsion E7.

concerns E7, it showed oil droplets with a diameter of $6.89 \pm 0.29 \mu\text{m}$, which were significantly larger than E4, but did not show any significant difference with E2. The CLSM images (Fig. 2, images D, E, and F) showed a more homogenous distribution of oil droplets (stained in red) in the emulsions E2 and E4 than E7, in addition in E2 and E7 a beginning of coalescence phenomena could be observed, leading to an increase of particle diameter and less regular shape of oil droplets as it was observed by Espert et al. (2017) in the study of cellulose-based emulsion production. Indeed, lower droplet diameter of oil globules could favour Brownian motion effects determining a homogeneous distribution of droplets, which are able to counteract the gravitational forces (Olsson

et al., 2018). Several authors assessed a negative correlation between the solid particle concentration and the oil droplets dimension, in fact a thick layer of solid particles at the interface of the oil droplet inhibits their fusion, determining a smaller size (Frelichowska et al., 2010; Song et al., 2022; Huc-Mathis et al., 2020). Burgos-Díaz et al. (2022) demonstrated that the use of particles from agri-food by-products significantly improved the performance of Pickering emulsification, resulting in smaller droplet sizes at higher particle concentrations compared to lower concentrations. Similarly, Fen et al. (2020) found that large amount of food grade gelatine nanoparticles facilitated the formation of smaller droplets by reinforcing the formation of interface

film and network structure, thereby preventing further coalescence. Furthermore, [Huc-Mathis et al. \(2020\)](#) not only correlated smaller droplet sizes with a higher concentration of solid particles but also demonstrated a slight influence of oil content, with a Pearson coefficient of -0.31 found between droplet diameter and oil content. An opposite trend was observed by [Zheng et al. \(2020\)](#), indeed, the increasing oil content in sea buckthorn oil-in-water emulsions, stabilized by different emulsifiers (sodium caseinate, soy protein isolate and sugar ester) caused a significant increase in droplets size. Likewise, an increase of Z-average diameter was found with the increase of the oil-phase volume fraction in the emulsion prepared with flaxseed protein concentrate containing mucilage, suggesting an insufficient absorption layer and the formation of flocculated droplets ([Wang et al., 2010c](#)).

Moreover, through the cryo-SEM analysis it was possible to focus on the original gel matrix structure due to the water in a glassy state rather than crystalline ([Sriamornsak, Thirawong, Cheewatanakornkool, Burapapadh, & Sae-Ngow, 2008](#)). As shown in Fig. 2 G, H and I, E4 exhibited a denser structure compared to E2, where the presence of a well-reticulated network was clearly visible. The observed difference in the E4 sample, containing 30% oil, could be attributed to its higher oil content, resulting in a firmer structure, as indicated by the textural results. These findings align with the results of cryo-SEM experiments conducted by [Zhao et al. \(2022\)](#) on starch-based emulsion gels. Their research revealed that emulsions containing 50% and 70% oil exhibited a denser structure and smaller pores compared to emulsions with 0–30% oil fraction. As regard sample E7, it showed a dense and compact continuous phase attributable to the large amount of ff-DWOC and oil, and low content of water.

3.2.4. Rheological analysis

The effect of frequency sweep test on the viscoelastic properties of the emulsion gels are represented in Fig. 3. The storage modulus (G') is a measure of the energy stored per cycle of deformation and reflects the elastic or solid-like behaviour, while the loss modulus (G'') is related to the fluid-like or viscous characteristics of the material ([Erçelebi and İbanoglu, 2009](#); [Román et al., 2015](#); [Yang et al., 2013](#)). Considering the obtained results, we can state that the three emulsions (E2, E4, and E7) exhibited a gel-like structure with greater values of storage modulus (G') than the loss modulus (G''), and showed a frequency dependence, which is a typical behaviour of weak gels ([Geremias-Andrade, Souki, Moraes, & Pinho, 2016](#); [Mun et al., 2009](#)). The emulsion E4, which was constituted by 25% of ff-DWOC and 30% of oil, showed the highest G' and G'' values

Table 6

Rheological properties of emulsions at the frequency of equal to 1 Hz.

Sample	G'	G''	$\tan \delta$
E2	$512.55 \pm 16.25c$	$126.65 \pm 3.55 b$	$0.25 \pm 0.00a$
E4	$3116.00 \pm 161a$	$584.8 \pm 24.4a$	$0.18 \pm 0.00c$
E7	$805.9 \pm 42.5 b$	$156.55 \pm 6.55 b$	$0.19 \pm 0.00 b$

Different letters in the same column mean significant differences (p -value < 0.05) according to one-way ANOVA and the Tukey's HSD test.

(Table 6), with a G' significantly higher than G'' , suggesting a marked solid-like behaviour in line with the results obtained by [Bascuas, Herando, Moraga, and Quiles \(2019\)](#). This result could be correlated with the smaller size of the oil droplets and, consequently, the supposed higher volume fraction, as observed in the microstructure analysis. Therefore, the increased density of oil droplets would promote the formation of cross-links per unit volume, enhancing the gel strength ([Xi, Liu, McClements, & Zou, 2019](#)). Also, E2 and E7 showed values of $G' > G''$, but significantly lower than the two moduli of E4. Furthermore, the difference between the two moduli was less pronounced compared to what was observed for E4, assuming a less elastic emulsion and greater weakness of the gel ([Ma, Liu, Bao, Liu & Zhang, 2013](#)), corroborating the textural results. From the obtained results, we could state that solid particles had a great influence on the viscoelastic properties of the emulsions. In fact, [Rudra et al. \(2020\)](#) and [Ketenoglu et al. \(2014\)](#) observed an increase in G' and G'' proportional to the concentration of pea pod powder (5–10%) and dietary fibre (1–3%) added during emulsion production. Additionally, the oil content also has a significant effect on the rheology of the emulsions as assessed by several studies ([Dickinson, 2012](#); [Ma & Boye, 2012](#); [Xi et al., 2019](#); [Zhao, Li, Wang & Wang, 2022](#)). In line with our results, [Ma et al. \(2013\)](#) observed that increasing the amount of oil allows the production of salad dressing supplemented with lentil flours with an instant elastic behaviour. However, the effect of the oil seems to be correlated with its quantity, as it can act as an active filler (strengthening effect) or an inactive filler (destructive effect) ([Zhao et al., 2022](#); [Dickinson, 2012](#)). [Zhao et al. \(2022\)](#) noted that in the sample with 10% the oil acted as an inactive filler, resulting in a weaker emulsion gel compared to the control that had no oil. On the contrary, starting from 20% of oil, the emulsions exhibited a more elastic behaviour, as the oil acted as active filler reinforcing the emulsion structure.

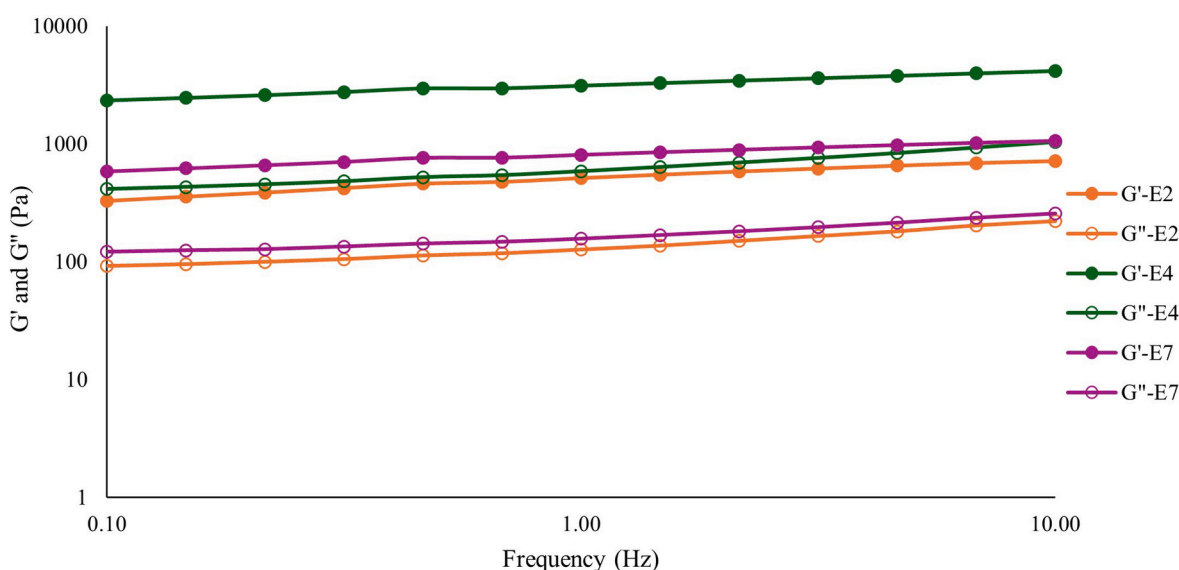


Fig. 3. Trend of storage modulus (G' , Pa) and loss modulus (G'' , Pa) of emulsions: E2, E4 and E7.

4. Conclusions

In this study, the potential of utilizing ff-DWOC and durum wheat oil for the production of physically stable emulsions was assessed. The nine emulsions exhibited a high variability of their textural characteristics as the proportions of the ingredients used were altered, in accordance with DoE model. The results obtained from the analysis of creaming index and texture revealed intriguing relationships between the stability and texture of the emulsions and the varying amounts of solid particles and oil content. Among the nine emulsions, it was evident that E2, E4, and E7 displayed superior stability and texture values compared to the others. LM, CLSM, and Cryo-FESEM images were used to examine the morphology of the three emulsions (E2, E4, and E7). The three emulsions showed a fine and compact structure, specifically E4 had the smallest droplets and well reticulated network, contributing to the firmer structure. The rheological analysis demonstrated that all the three emulsions exhibited a gel-like behaviour, corroborating the results obtained in terms of stability and textural properties. Although the emulsions showed promising stability over the 14 days storage period, longer-term stability studies, under various conditions, may be needed to assess their suitability for commercial applications where extended shelf-life is required. Concluding, these emulsions have exhibited promising characteristics, making them viable candidates for utilization within the food industry as innovative ingredients. In a future perspective, the investigation about scaling up the production to industrial level is crucial, which may involve pilot-scale trials, assessing the scalability of the process without compromising the quality of the product. Moreover, assessing the environmental impact and finding greener alternatives the ingredients used in the emulsion is essential to enhance the overall sustainability of the product.

CRedit authorship contribution statement

Giuditta de Gennaro: Writing – original draft, Software, Methodology, Investigation. **Graziana Difonzo:** Writing – review & editing, Writing – original draft, Supervision, Software, Methodology, Investigation, Conceptualization. **Amparo Quiles:** Writing – review & editing, Writing – original draft, Supervision, Software, Methodology, Investigation. **Isabel Hernando:** Writing – review & editing, Supervision, Conceptualization. **Francesco Caponio:** Writing – review & editing, Supervision.

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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