POSTER COMMUNICATIONS

**Advanced Emulsions as Bioactive Compound Carriers for Functional Food Design: Technological and Nutritional Aspects**

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Four types of emulsions (i.e., single, cold filled-gel, hot filled-gel, and Pickering) were produced and characterized to develop carriers for polyphenols extracted from olive-vegetative water. Central Composite Designs were applied to study the effects of different emulsifier types and concentrations, and oil phase percentages. After the technological characterization of all the samples, Response Surface Methodology was applied to obtain optimized emulsion formulations for the future encapsulation of polyphenols and food enrichment applications.

**Emulsioni avanzate come sistemi di trasporto di composti bioattivi per lo sviluppo di alimenti funzionali: aspetti tecnologici e nutrizionali**

Quattro tipologie di emulsioni sono state studiate come possibili *carrier* per polifenoli derivanti dalle acque di vegetazione delle olive: singole, gelificate a freddo, gelificate a caldo e Pickering. L’applicazione di *Central Composite Designs* ha permesso di studiare l’effetto delle differenti tipologie di emulsionanti, della concentrazione di emulsionante e di diverse percentuali di fase oleosa. Attraverso la metodica delle superfici di risposta, sono state ottimizzate le formulazioni delle emulsioni da utilizzare in futuro per l’incapsulamento di polifenoli e lo sviluppo di alimenti arricchiti.

**Keywords:** Pickering emulsion, filled-gel emulsion, single emulsion, Design of Experiment, Response Surface Methodology.

# **1. Introduction**

The PhD project is about the development of lipid carriers for natural polyphenol extract obtained from olive-vegetative water, for future food reformulation to promote sustainability and healthiness. Here, the main results derived from the first activities of the project are shown:

(A1) Design of different types of advanced emulsions by applying Design of Experiment (DoE) techniques to study the main and interaction effects of type of emulsions, concentration of emulsifiers, and concentration of corn oil, and characterization of emulsions for the most relevant technological parameters.

(A2) Optimization of the different emulsion formulations by applying the Response Surface Methodology (RSM).

# **2. Materials and Methods**

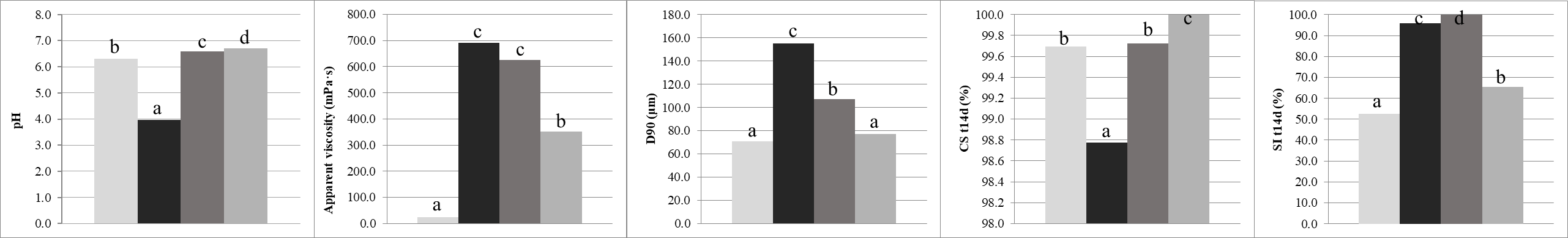
Four different oil-in-water emulsions were studied: single, cold filled-gel, hot filled-gel, and Pickering emulsions. By applying a face-centred central composite design (Design Expert, v. 10.0.0.3, Stat-Ease Inc., MN, USA), thirteen runs for each kind of emulsion were developed and characterized. Lecithin (Le; 0.5-1.5-2.5% in oil) and citrus fibres (CF; 4-6-8% in water) were tested as emulsifiers for single and cold filled-gel emulsions, respectively; chickpea protein isolate (CP; 8-12-16% in water) was used as emulsifier in both hot filled-gel and Pickering emulsions. Corn oil was studied at 15-37.5-60% levels in all the emulsions. After mixing the emulsifier, the water and the oil phase were homogenized with a high-shear homogenizer (UltraTurrax T25, IKA, Germany) at 21000 rpm for two cycles of 30 s, with a resting phase of 30 s. All samples were analysed for pH (SevenEase, Mettler Toledo S.p.A., Italy), particle size distribution (Mastersizer 3000, Malvern Panalytical, UK), apparent viscosity (MCR 102 rheometer, Anton Paar, Austria), and stability at 4°C for 14 days. Moreover, optical (mod. B1000 with digital camera, Optika Microscopes, Italy) and confocal laser scanning (Nikon A1, Nikon, Netherlands) microscopy observations were performed. All the results were statistically analysed with a Multifactor Analysis of Variance (MANOVA) followed by the Least Significant Difference (LSD) test to identify the significant main and interaction effects (i.e., A. emulsion type; B: emulsifier concentration; C: corn oil concentration; AB, AC, BC: two-way interactions). The Pearson correlation matrix (Statgraphics Centurion 18, v. 18.1.13) was also studied, to identify significant correlations between couples of variables. At last, RSM (Design Expert, v. 10.0.0.3, Stat-Ease Inc., Minneapolis, MN, USA) was applied for the optimization of the four emulsion formulations.

# **3. Results and Discussion**

## **3.1 Main and interaction effects evaluation**

Microscopy observations confirmed the different structure of the advanced emulsions, revealing the adsorption of CP at the oil droplet interfaces in Pickering emulsions, while a 3D-network was created by gelled CP in hot-gel filled emulsions. A 3D-matrix was visible also in cold-gel filled emulsions produced with CF, due to their ability to entrap water. All the parameters evaluated on emulsions were significantly affected (p<0.001) by the main experimental factors and their interactions. For the sake of brevity, only results about the type of emulsions are here commented and graphically showed (Fig. 1). In cold-filled gel emulsions, the lowest pH average values (3.97) and the highest average apparent viscosity levels (684 mPa·s) were determined. A similar apparent viscosity was obtained in hot-filled gel emulsions made with CP (627 mPa·s). The lowest average particle size (D90) was obtained in Pickering emulsions (77 µm) produced with CP in cold conditions. Consequently, Pickering emulsions showed the highest creaming stability (CS) after 14 days of storage (99.9%), while cold-filled gel emulsions had the lowest CS (98.8%) related to the highest oil droplet size (155 µm). As for stability index (SI), an interesting result was obtained using CP in the two different emulsions: SI was at maximum levels in hot-filled gel emulsions (99.5%), but at the minimum in Pickering emulsions (67%). These results can be related to the gelation capacity of chickpea proteins when heated (Karaca et al., 2011). With respect to the advanced emulsions, single emulsions made with lecithin showed in average a very low apparent viscosity (14.6 mPa s) and a low SI (52%). From the Pearson correlation matrix, a negative correlation was found between pH, apparent viscosity, and the particle dimensions, which had also a negative correlation with CS; this might be motivated by the fact that with the increasing of particle dimensions, instability phenomena (such as coalescence) are more frequent, promoting oiling off; a higher apparent viscosity, on the contrary, can promote a more compact structure in which the oil droplets are less prone to move and coalesce (McClements, 2015).

**Figure 1** *MANOVA results for emulsion types (bars from left to right represent single, cold-filled gel, hot-filled gel, and Pickering emulsions; bars with different letters show significantly different results).*



## **3.2 Optimization with RSM technique**

RSM results showed highly significant models for all the response variables (p<0.0001), except for pH (p<0.05), CS (p<0.05), and SI (not significant). Thus, the optimization was carried out with a desirability function that maximized the apparent viscosity and minimized the droplet size dimension (D90). In table 1 the optimized formulations that met all the criteria to develop a functional and stable delivery system are summarized.

***Table 1*** *Optimized formulations for the four types of studied emulsions.*

|  |  |  |  |
| --- | --- | --- | --- |
| **Emulsion type** | **Emulsifier type** | **Emulsifier (%)** | **Oil (%)** |
| Single | Lecithin | 2.5 | 58.49 |
| Cold filled-gel | Citrus fibres | 8.0 | 40.66 |
| Hot filled-gel | Chickpea protein isolate | 16.0 | 60.00 |
| Pickering | Chickpea protein isolate | 16.0 | 60.00 |

In table 2 results of technological parameters for the optimized emulsions are reported. pH of CF\_cold\_OPT resulted significantly lower, and this might have affected CS and SI, as well as the droplet size (Qi et al., 2021). The significant highest apparent viscosity of CP\_hot\_OPT is related to their ability to gelate and produce stable emulsions, with also small droplet size (Karaca et al., 2011).

***Table 2*** *Results of the optimized emulsion characterization.*

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
|  | **Optimized emulsion samples** | | | |
| **Parameters** | **Le\_sing\_OPT** | **CF\_cold\_OPT** | **CP\_hot\_OPT** | **CP\_Pick\_OPT** |
| *pH* | 6.354 ± 0.205b | 3.906 ± 0.022a | 6.597 ± 0.013c | 6.635 ± 0.004c |
| *Apparent viscosity (mPa·s)* | 144 ± 12a | 1365 ± 46b | 2380 ± 123d | 1868 ± 57c |
| *D90 (μm)* | 19 ± 1a | 139 ± 6d | 83 ± 9c | 45 ± 8b |
| *CS t14d* | 100.0 ± 0.1b | 99.8 ± 0.1a | 100.0 ± 0.1b | 100.0 ± 0.1b |
| *SI t14d* | 88.7 ± 0.4a | 97.6 ± 0.1 b | 100.0 ± 0.1c | 100.0 ± 0.1c |

# **4. References**

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