

Multivariate data analysis for the development of multiple emulsions based on cold plasma treated sunflower oil

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Abstract – The properties of vegetable oils can be improved by initiating their polymerization, in order to increase their viscosity, their thermal stability and their drying capacity. A process based on cold plasma treatment represents mild and ecofriendly conditions to get pre-polymerized vegetable oils. A pre-polymerized sunflower vegetable oil was chosen to prepare water-in-oil-in-water emulsions, using an association of biobased surfactants. Emulsification conditions were optimized using central composite design (CCD) and principal component analysis (PCA) based on temperature (25–50 °C), casein content (1.5–3.5% w/w), and alkylpolyglucoside (APG) concentration (1.5–3.5% w/w). Evaluated responses were the onset of destabilization (OOD), creaming index (CI), and droplet sizes. CCD results suggest an optimum at temperature of 35 °C using 3.0% of APG and 1.8–3.9% of casein, yielding lower water droplet diameters ensuring emulsion stability. Furthermore, PCA results highlighted a positive correlation between ODD and the total amount of surfactant (Pearson correlation coefficient = 0.67), and a negative correlation between CI and the total amount of surfactant (Pearson correlation coefficient = -0.62). Casein rate and temperature of emulsification are the factors which control the inner water droplet sizes of the studied system, while total amount of surfactant impacts the ODD and CI.

Keywords: Emulsion / pre-polymerized vegetable oil / central composite design / principal component analysis / stability

Résumé – **Analyse de données multivariées pour le développement d'émulsions multiples à base d'huile de tournesol prétraitée sous plasma.** Les propriétés des huiles végétales peuvent être améliorées en initiant leur polymérisation, afin d'augmenter leur viscosité, leur stabilité thermique et leur capacité de séchage. Un processus basé sur le traitement par plasma froid représente des conditions douces et écologiques pour obtenir des huiles végétales pré-polymérisées. Une huile végétale de tournesol pré-polymérisée a été choisie pour préparer des émulsions eau-dans-huile-dans-eau, en utilisant une association de tensioactifs biosourcés. Les conditions d'émulsification ont été optimisées à l'aide d'un plan composite centré et d'une analyse en composantes principales (ACP) basée sur la température (25–50 °C), la teneur en caséine (1,5–3,5% m/m) et la concentration en alkylpolyglucoside (APG) (1,5–3,5% m/m). Les réponses évaluées étaient le début de la déstabilisation (OOD), l'indice de crémage (IC) et la taille des gouttes. Les résultats du plan suggèrent un optimum à la température de 35 °C en utilisant 3,0% d'APG et 1,8-3,9% de caséine, correspondant à des diamètres de gouttelettes d'eau plus faibles assurant la stabilité de l'émulsion. En outre, les résultats de l'ACP ont mis en évidence une corrélation positive entre l'OOD et la quantité totale de tensioactif (coefficient de corrélation de Pearson = 0,67), et une corrélation négative entre l'IC et la quantité totale de tensioactif (coefficient de corrélation de Pearson = -0.62). Le taux de caséine et la température d'émulsification sont les facteurs qui contrôlent la taille des gouttelettes d'eau internes du système étudié, tandis que la quantité totale de tensioactif a un impact sur l'OOD et l'IC.

Mots-clés : Emulsion / huile végétale prépolymérisée / plan composite centré / analyse en composantes principales / stabilité

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Highlights

- W/O/W emulsions based on a pre-treated sunflower oil were developed using two biobased emulsifiers.
- Emulsion conditions were optimized using central composite design (CCD) and principal component analysis (PCA).
- CCD suggested an optimum in temperature and emulsifier content for emulsion stability.
- PCA highlighted correlations between experimental parameters.

1 Introduction

Vegetable oil-based emulsions are used in various formulations in food, cosmetics and lubricants industries, in addition to their application in biocontrol (Tadros, 2016). However, emulsions can exhibit thermodynamical instability during storage, resulting in unwanted effects such as creaming, sedimentation, flocculation, or coalescence (Sjöblom, 2006). Vegetable oil-based emulsions are usually formed using oils with different fatty acid profiles depending on the targeted application. For instance, unsaturated vegetable oils are exploited for their drying properties, due to which, they are applied in coatings. The quality of emulsions can be improved by optimizing the oxidative and thermal stability of the oil used while increasing their viscosity. Therefore, vegetable oils are sometimes replaced by their pre-polymerized forms, such as stand oils, blown oils or boiled oils, prepared by heating the natural oils in the presence of a catalyst. For instance, emulsions based on stand oils have been used in eco-friendly paints (Alouche *et al.*, 2005). Due to their ease of film-forming, polymerized oils result in harder and faster-drying films as compared to their non-polymerized counterparts (Nunes *et al.*, 2020; Seniha Güner, Yağcı, et Tuncer Erciyaset al., 2006, Viani, et Ševčík). Polymerization of vegetable oils can be carried out through alternative metal-free processes based on a dielectric activation (microwave, plasma). Among these processes, the cold plasma technology consists of applying electrical discharges to a gas, between two metal electrodes, to create plasma. According to Godfroid *et al.* (2023), plasma species (a mixture of ions, electrons and radicals) promote oligomerization and polymerization of poly-unsaturated triglycerides and generate cross-linking in the natural oil. This process can also lead to the formation of conjugated double bonds in the fatty acid chains, followed by dimerization through Diels-Alder reaction (Yepez *et al.*, 2021). This technology has been patented and applied to vegetable oils used in cosmetic applications and in food industry (Danneaux and Le Bihan, 2024). Nevertheless, a reduced plasma exposure time is suggested to preserve food bioactive molecules (Afshar *et al.*, 2022). The cold plasma treatment is also reported to limit the formation of *trans* fatty acid during hydrogenation (Yepez and Keener, 2016; Puprasit *et al.*, 2020). Furthermore, such cross-linked vegetable oils act as rheological modifiers and serve to increase the lubricity of fuels (Absil and Danneaux,

2021; Godfroid *et al.*, 2020). Recent works have reported the preparation of plasma polymerized vegetable oils to design hydrophobic papers (Bellmann *et al.*, 2024). In this context, the objective of the present study is to develop biobased W/O/W emulsions from sunflower oil modified by partial hydrogenation under cold plasma. Optimal process parameters for formulation were determined from central composite design (CCD) and principal component analysis (PCA) was used to highlight correlations between the different variables.

2 Materials and methods

Pre-polymerized sunflower oil was produced by a plasma activation process under hydrogen flow as described by Godfroid *et al.* (2023). The resulting oil has the following characteristics: density at 20 °C of 0.929 g/cm³, iodine value of 100.5 g/100 g, peroxide value of 9.7 meq/kg, acid value of 1.88 mg KOH/g, saponification value of 192 mg KOH/g and a portion of unsaponifiable matter of 0.53%. The kinematic viscosity was measured to be 1973 mm²/s at 40 °C.

The biobased surfactants tested were SimulsolTM SL26C (51%) (alkylpolyglucoside, APG) and MontaneTM 20 (99.84%) (sorbitan ester), which were supplied by Seppic (France). Appyclean 6552 (60%) (alkylpolypentosides, APP) was obtained from Wheatoleo (France). Casein from bovine milk (> 95%), used as a co-emulsifier, was purchased from Fluka Biochemika. Sodium hydroxide pellets (97%) were purchased from Sigma Aldrich.

2.1 Selection of surfactants

Three biobased surfactants were selected based on their range of Hydrophilic-Lipophilic (HLB) values which are suited to the formulation of oil-in-water emulsions. Casein was chosen as a possible co-emulsifier (Tab. 1). APG and APP can be synthesized by Fischer's glycosylation involving the reaction between a monosaccharide and a fatty alcohol (Sangiorgio *et al.*, 2022). As for the sorbitan ester, it can be obtained by esterification or alkali-catalyzed transesterification of sorbitol and a fatty acid (Balzer and Lüders, 2000).

Three emulsions were prepared as followed: 0.24 g of APG (or 0.13 g of sorbitan ester or 0.21 g of APP) was added to 2 g of pre-polymerized sunflower oil. The aqueous phase (2 g of water) was added dropwise to the lipophilic phase under magnetic stirring (150 rpm) at 35 °C. 4 g of emulsion were thus obtained with the following composition: 3% (w/w) of emulsifier, 48% (w/w) of pre-polymerized sunflower oil and 49% (w/w) of demineralized water.

A fourth emulsion was prepared by adding the emulsifier (0.13 g of casein) in 2 g aqueous phase including 0.01 g of a 30% (w/w) sodium hydroxide solution. Then this solution was added dropwise to the lipophilic phase (2 g) under magnetic stirring (150 rpm) at 35 °C.

Then, four other emulsions including casein were prepared as followed: 0.24 g of APG (or 0.13 g of sorbitan ester or 0.21 g of APP) were added to 4 g of pre-polymerized sunflower oil, while 0.13 g of casein was solubilized in 4 g of aqueous phase with 0.02 g of a 30% (w/w) sodium hydroxide solution. The aqueous phase containing casein was added dropwise to the lipophilic phase under magnetic stirring (150 rpm) at 35 °C. 8 g

Table 1. Characteristics of emulsifiers selected.

Surfactant	HLB	Origin
APG	12	Glucose + fatty alcohol
APP	11.5-12	Pentose + fatty alcohol
Sorbitan ester	8.6	Sorbitol + fatty acid (or methyl ester)
Casein	-	Bovine milk

of emulsion were thus obtained containing: 3% (w/w) of a combination of a surfactant (APG, sorbitan ester or APP) and casein (ratio 1:1), 48% (w/w) pre-polymerized sunflower oil and 49% (w/w) demineralized water.

The emulsifier performances were then compared based on the macroscopic stability of the emulsion, defined by the appearance of creaming after one day.

2.2 Preparation of emulsions

17 emulsions containing casein (1.5–3.5% w/w), alkylpolyglucoside (APG) (1.5–3.5% w/w) and demineralized water were prepared at different temperatures (25–50 °C) based on the three-factor central composite design (CCD). The emulsions were formulated using the phase inversion technique. The lipid phase was a combination of 48% (w/w) pre-polymerized sunflower oil with alkylpolyglucoside, to which demineralized water with solubilized casein was added at the rate of 0.5 mL/min under continuous stirring (150 rpm) with an emulsifying stirrer (ø42 mm, IKA, Germany).

2.3 Stability of emulsions

The stability of the emulsions was determined using Turbiscan Classic 2 (Microtrac-Formulation, France). A known volume (7 mL) of the emulsion sample was placed in a cylindrical glass cell. Emulsion destabilization was analyzed simultaneously from transmission (*T*) and backscattering (*BS*) profiles. Emulsion samples were scanned at 880 nm using the following protocol: 1 scan every min for 1 h, every 5 min for 2 h, every 10 min for 9 h and each day for 14 days. The emulsion stability was assessed by determining the onset of destabilization (OOD, hour), defined as the moment when the transmission reached 0.2% and by the creaming index (CI, %) obtained after 14 days of storage. Both the parameters were calculated using Turbisoft-Classical 2 software (version 2.3.1.125) of Turbiscan Classic 2 (Microtrac-Formulation, France),

2.4 Optical microscopy and droplet size measurements

The microstructures of the emulsions were characterized by optical microscopy (Nikon Eclipse E600, Japan) connected to a digital camera (Nikon DS-Fi2, Japan). A drop of the emulsion was placed on a microscope slide, covered with a coverslip, and observed at x 400 magnification. NIS-Elements

Table 2. Matrix of the central composite design (CCD) with X_1 : Temperature (°C), X_2 : Amount of casein (% (w/w)) and X_3 : Amount of alkylpolyglucosides (% (w/w)).

Experimental code	Transformed Values		
	X_1	X_2	X_3
LB01039	-1	-1	-1
LB01038	1	-1	-1
LB01047	-1	1	-1
LB01048	1	1	-1
LB01043	-1	-1	1
LB01042	1	-1	1
LB01051	-1	1	1
LB01040	1	1	1
LB01036	-1.35	0	0
LB01037	1.35	0	0
LB01046	0	-1.35	0
LB01049	0	1.35	0
LB01045	0	0	-1.35
LB01044	0	0	1.35
LB01034	0	0	0
LB01052	0	0	0
LB01050	0	0	0

Coded Values	Actual Values		
	X_1	X_2	X_3
-1.35	20.6	1.1	1.1
-1	25	1.5	1.5
0	37.5	2.5	2.5
1	50	3.5	3.5
1.35	54.4	3.9	3.9

software was used to determine droplet diameters. Size distributions were characterized by the mean diameters of the particle based on the volume-weighted mean diameter (De Brouckere diameter, D_{43}), area-weighted mean diameter (Sauter diameter, D_{32}), and the number-weighted mean diameter (M) according to the following equations:

$$D_{43} = \frac{\sum n_i d_i^4}{\sum n_i d_i^3}, \quad (1)$$

$$D_{32} = \frac{\sum n_i d_i^3}{\sum n_i d_i^2}, \quad (2)$$

$$M = \frac{\sum n_i d_i}{\sum n_i}. \quad (3)$$

With the number of droplets being n_i and their diameters being d_i .

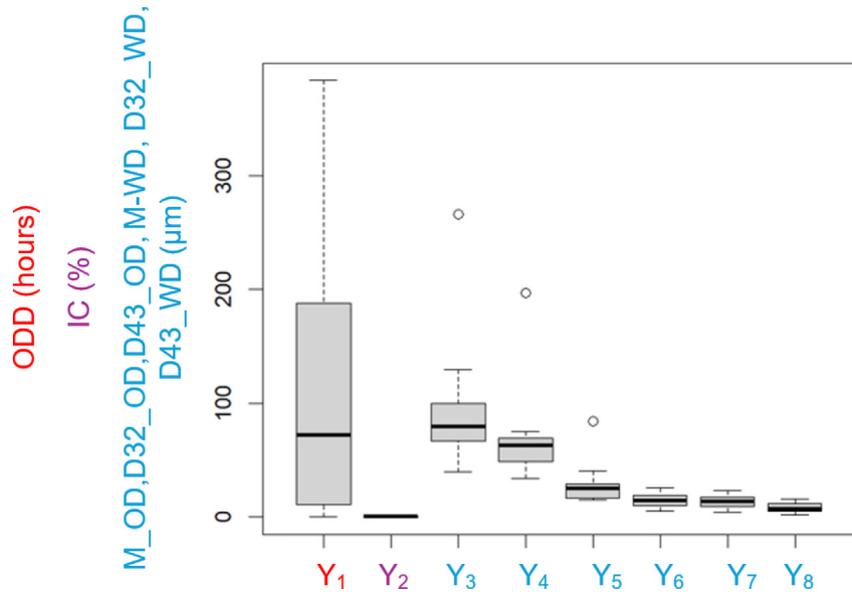


Fig. 1. Boxplot for the determination of outliers.

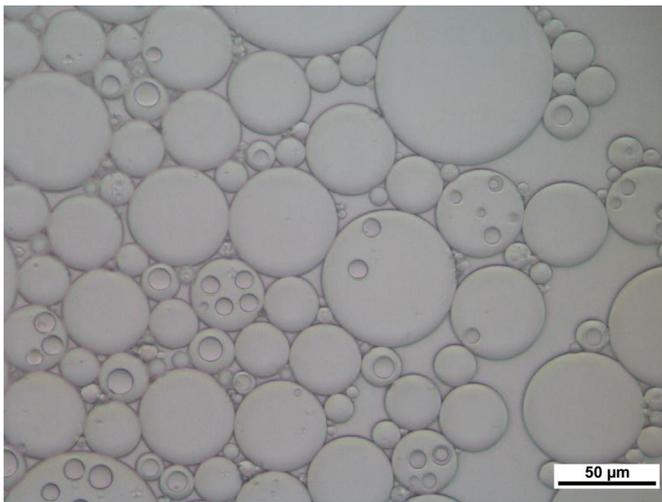


Fig. 2. Micrography of a W/O/W emulsion based on cold plasma-treated vegetable oil (x 400) (LB01046).

2.5 Experimental design and statistical analysis

A five-level three-factor central composite design ($\alpha = 1.35$) was used to evaluate the influence of the different variables [temperature (X_1), amount of casein (X_2), and amount of alkylpolyglucoside (X_3)] on the stability of the emulsions. The design was composed of eight factorial points, six axial points and three centred points, with a total of 17 runs (Tab. 2). The responses of the CCD were i) the average droplet size of the oil particles (D43_OD, D32_OD, M_OD), ii) the average droplet size of the water particles in the oil (D43_WD, D32_WD, M_WD), iii) the creaming index after storage for 14 days (CI, %), and iv) the onset of destabilization (OOD, hour). A second-order quadratic model, including interaction and polynomial terms was employed to evaluate the responses:

$$Y_i = a_0 + a_1X_1 + a_2X_2 + a_3X_3 + a_{12}X_{12} + a_{13}X_{13} + a_{23}X_{23} + a_{11}X_1^2 + a_{22}X_2^2 + a_{33}X_3^2. \quad (4)$$

Data processing was carried out using the software RStudio (version 2023.12.1-402). Regression analysis of the data was performed using the package “rsm”. Statistical significance was set as $p < 0.05$ to validate the fit of the model for each response to the experimental data. Outliers were determined using a box plot, with the first and third quartiles (Q1 and Q3) of the data calculated. The interquartile range (IQR) was then determined by subtracting Q1 from Q3 (IQR = Q3 - Q1). The upper and lower limits of the outliers were calculated as $Q3 + 1.5 \times IQR$ and $Q1 - 1.5 \times IQR$, respectively. Any data point that falls above the upper limit or below the lower limit is considered an outlier. These outliers are typically visualized as individual points outside the box plot (Fig. 1).

Principal Component Analysis (PCA) was performed using the package “Factoshiny” on RStudio software (version 2023.12.1-402). Contour and response surface plots were drawn to analyze the variations in response relative to the variables. Pearson correlation coefficients (PCC) were determined to express the correlation between each variable. A high PCC (> 0.60) means a high correlation between variables. The \cos^2 of the angle between a principal component and a variable is calculated by the RStudio software. \cos^2 represents the quality of the representation of a variable in a principal component. A higher \cos^2 value means that the variable is well represented by the component, while a lower value suggests a weaker representation.

3 Results and discussion

3.1 Screening of surfactants

Seven emulsions were prepared using either a single surfactant or an association of a surfactant and a co-emulsifier. The stabilities of these emulsions were evaluated as presented in Table 3.

Table 3. Characteristics of the formulated emulsions.

N° emulsion	Surfactant	Emulsifier	Stability
E1	APG (3% w/w)	-	Destabilization after a few minutes
E2	APP (3% w/w)	-	Destabilization after a few minutes
E3	Sorbitan Ester (3% w/w)	-	Destabilization after a few minutes
E4	-	Casein (3% w/w)	Destabilization after a few minutes
E5	APG (1.5% w/w)	Casein (1.5% w/w)	Destabilization after 1 day
E6	APP (1.5% w/w)	Casein (1.5% w/w)	Destabilization after a few minutes
E7	Sorbitan Ester (1.5% w/w)	Casein (1.5% w/w)	Destabilization after a few minutes

Table 4. Results of Central Composite Design. OOD: onset of destabilization, CI: creaming index, D43: De Brouckere diameter, D32: Sauter diameter, M: mean diameter, OD: oil droplets, WD: water droplets in oil drops.

Experimental codes	Y ₁ OOD (hour)	Y ₂ CI (%)	Y ₃ D43_OD (μm)	Y ₄ D32_OD (μm)	Y ₅ M_OD (μm)	Y ₆ D43_WD (μm)	Y ₇ D32_WD (μm)	Y ₈ M_WD (μm)
LB01039	0.1	31.7	266.6	196.9	84.5	17.4	16.5	13.4
LB01038	0.2	16.1	77.0	62.5	24.6	23.5	21.7	14.6
LB01047	24.0	19.6	98.6	70.2	28.6	10.9	10.0	5.1
LB01048	11.1	27.3	95.5	75.3	25.4	14.9	13.8	7.6
LB01043	73.4	5.7	66.7	38.9	16.2	11.5	10.5	6.3
LB01042	48.0	6.1	108.3	68.4	25.6	23.5	22.2	15.8
LB01051	384.1	0	99.9	67.6	16.0	6.8	6.0	3.2
LB01040	72.0	2.5	105.0	68.3	33.7	11.6	10.1	4.4
LB01036	9.3	14.1	49.9	35.0	14.8	25.4	22.9	10.6
LB01037	139.4	2.5	53.1	48.5	33.9	25.0	22.9	11.5
LB01046	55.7	17.3	71.3	55.7	26.8	9.8	9.2	5.8
LB01049	288.0	3.3	129.3	73.8	18.8	8.1	7.6	4.7
LB01045	2.7	22.6	83.6	69.1	40.3	18.6	17.4	11.4
LB01044	216.0	1.4	46.0	34.9	16.8	14.3	13.2	4.9
LB01034	187.9	1.3	39.5	34.0	20.6	14.5	13.6	9.5
LB01052	240.0	1.1	74.6	55.9	19.3	5.2	4.4	1.9
LB01050	144.0	19.1	79.4	59.7	15.8	5.4	5.0	3.7

Table 3 shows that the association of alkylpolyglucosides (APG) Simulsol™ SL26C and casein leads to the most stable emulsion as compared to that prepared with only casein, APG, or sorbitan ester. This result can be attributed to the formation of a surfactant/protein complex. Using a non-ionic surfactant minimizes protein aggregation, enabling the protein to be solubilized at the oil-water interface (Lee *et al.*, 2011). The surfactant/protein interactions are dependent on the concentration of the surfactant and its molecular structure (Aguirre-Ramírez *et al.*, 2021).

3.2 Central composite design

The above-described protocol leads to multiple emulsions with water droplets inside the oil drops, as shown in Figure 2.

Multiple emulsions have two thermodynamically unstable interfaces (Schmidts *et al.*, 2010). According to Schmidts *et al.* (2010), the ideal size of the inner droplet should be below 1 μm to improve the emulsion stability. It is thus interesting to study

the correlation between the sizes of the inner droplets and the stability of these multiple emulsions. The sizes of water droplets (responses Y₆ to Y₈) were measured to determine the influence of factors (X₁, X₂, X₃) on the multiplicity of emulsions (Tab. 4). Table 4 groups the results of the central composite design (CCD) for the 17 emulsions. The CCD led to emulsions with oil droplet sizes (D32_OD) ranging from 33.9 to 196.9 μm and inner water droplets sizes (D32_WD) ranging from 4.5 to 20.6 μm (Tab. 4).

Equations (5) to (9) have been determined by using the package “rsm” on RStudio and quantitatively represent the impact of the formulation variables on the measured responses (stability, creaming index and size distributions). The best models were retained for their high R², *ap* -value < 0.05 and a lack of fit with *ap* -value > 0.05.

$$Y_1(\text{OOD}) = 111.52 - 15.01X_1 + 58.66X_2 + 71.28X_3, \quad (5)$$

$$Y_2(\text{CI}) = 11.28 - 1.77X_1 - 2.50X_2 - 9.36X_3, \quad (6)$$

Table 5. Coefficients of the equations of Central Composite Design. OOD: onset of destabilization, CI: creaming index, D43: De Brouckere diameter, D32: Sauter diameter, M: mean diameter, OD: oil droplets, WD: water droplets in oil drops.

	Y ₁ OOD (hour)	Y ₂ CI (%)	Y ₆ D43_WD (μm)	Y ₇ D32_WD (μm)	Y ₈ M_WD (μm)
a ₀	111.52	11.28	10.55	9.69	7.91
significance	2.82E-4	9.33E-6	1.28E-3	1.55E-3	3.65E-7
a ₁	-15.01	-1.77	2.27	2.14	1.34
significance	0.59	0.38	0.12	0.12	0.21
a ₂	58.66	-2.50	-2.93	-2.85	-2.69
significance	0.05	0.22	0.05	0.04	0.02
a ₃	71.28	-9.36	-1.65	-1.62	-1.70
significance	0.02	3.41E-4	0.25	0.23	0.12
a ₁₂	-	-	-	-	-
significance	-	-	-	-	-
a ₁₃	-	-	-	-	-
significance	-	-	-	-	-
a ₂₃	-	-	-	-	-
significance	-	-	-	-	-
a ₁₁	-	-	6.48	5.81	-
significance	-	-	4.58E-3	6.18E-3	-
a ₂₂	-	-	-2.44	-2.11	-
significance	-	-	0.20	0.24	-
a ₃₃	-	-	1.69	1.64	-
significance	-	-	0.37	0.35	-

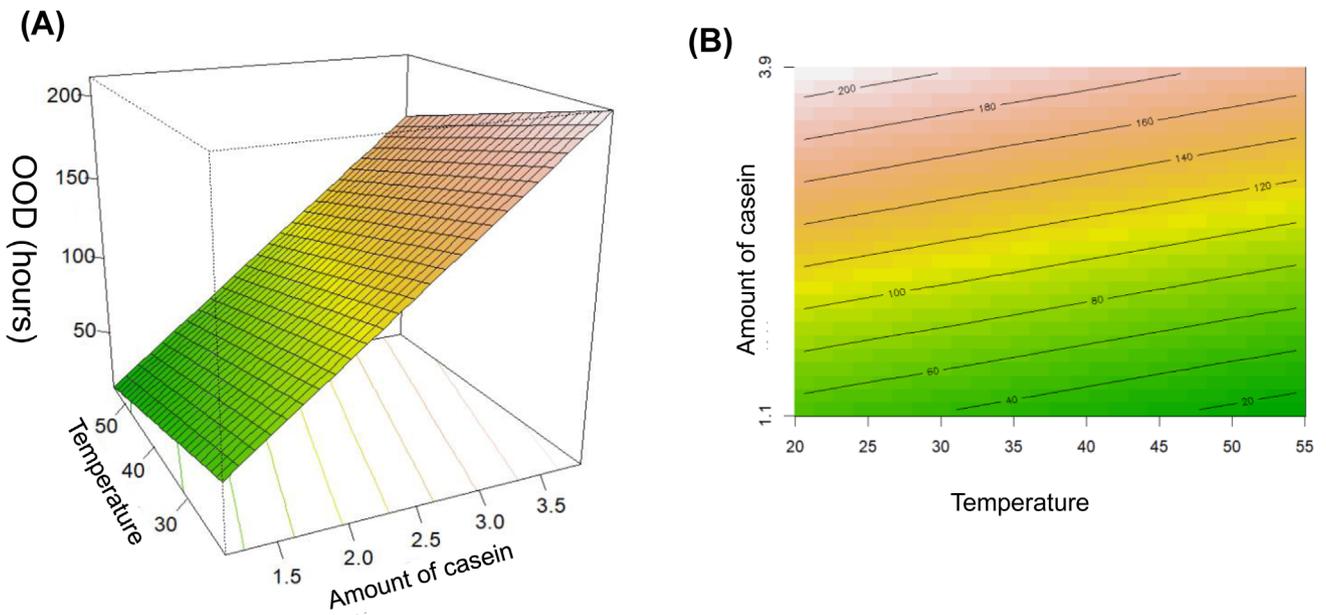


Fig. 3. Result of CCD: Influence of emulsification factors on OOD (Y₁) by response surface plot (A) Effect of X₁ and X₂ and contour plot (B) Effect of X₁ and X₂.

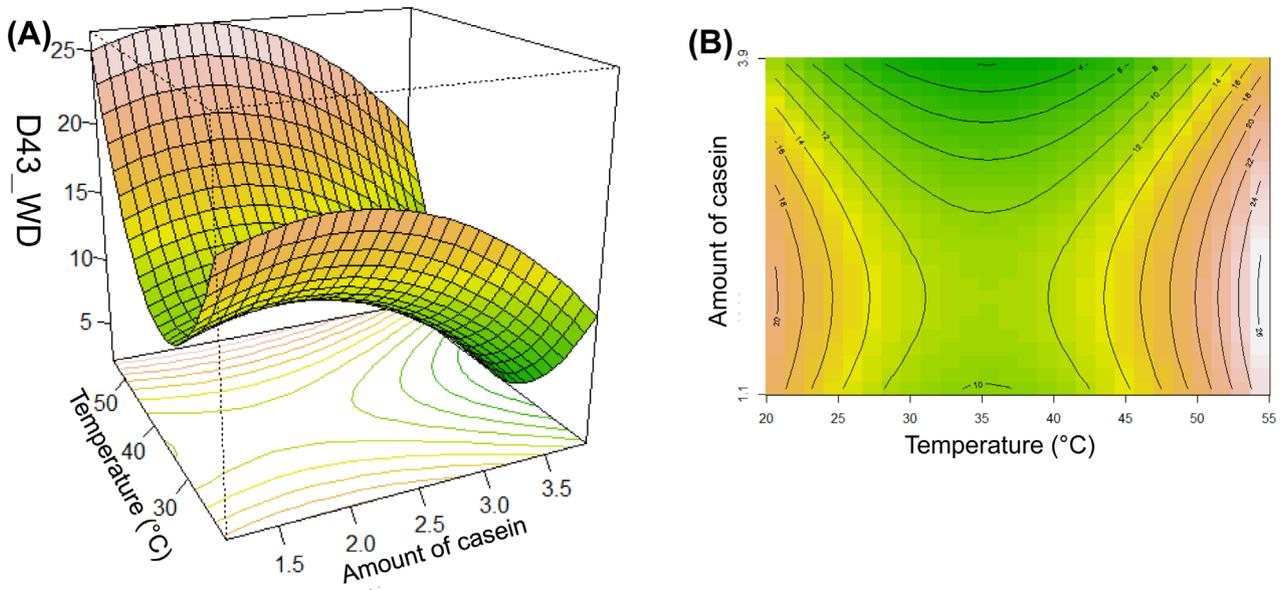


Fig. 4. Result of CCD: Influence of emulsification factors on D43_WD (Y_6) by response surface plot (A) Effect of X_1 and X_2 and contour plot (B) Effect of X_1 and X_2 .

Table 6. Coefficients of the equations of Central Composite Design without the outlier. OOD: onset of destabilization, CI: creaming index, D43: De Brouckere diameter, D32: Sauter diameter, M: mean diameter, OD: oil droplets, WD: water droplets in oil drops.

	Y_1 OOD (hour)	Y_2 CI (%)	Y_4 D32_OD (μm)	Y_6 D43_WD (μm)	Y_7 D32_WD (μm)
a_0	200.58	10.69	47.28	10.49	9.64
significance	2.76E-4	2.87E-5	1.18E-5	2.39E-3	2.85E-3
a_1	10.94	-0.92	5.22	2.11	2.02
significance	0.55	0.66	0.15	0.19	0.19
a_2	84.61	-1.65	6.91	-3.08	-2.97
significance	2.57E-3	0.43	0.06	0.07	0.07
a_3	97.23	-8.51	-5.72	-1.81	-1.74
significance	1.27E-3	1.20E-3	0.12	0.26	0.25
a_{12}	-75.24	-	-	-	-
significance	0.01	-	-	-	-
a_{13}	-78.34	-	-	-	-
significance	0.01	-	-	-	-
a_{23}	-0.27	-	-	-	-
significance	0.99	-	-	-	-
a_{11}	-76.21	-	-1.23	6.56	5.88
significance	9.15E-3	-	0.77	7.02E-3	9.42E-3
a_{22}	-22.72	-	11.38	-2.35	-2.05
significance	0.30	-	0.02	0.25	0.28
a_{33}	-57.01	-	4.38	1.78	1.70
significance	0.03	-	0.31	0.37	0.37

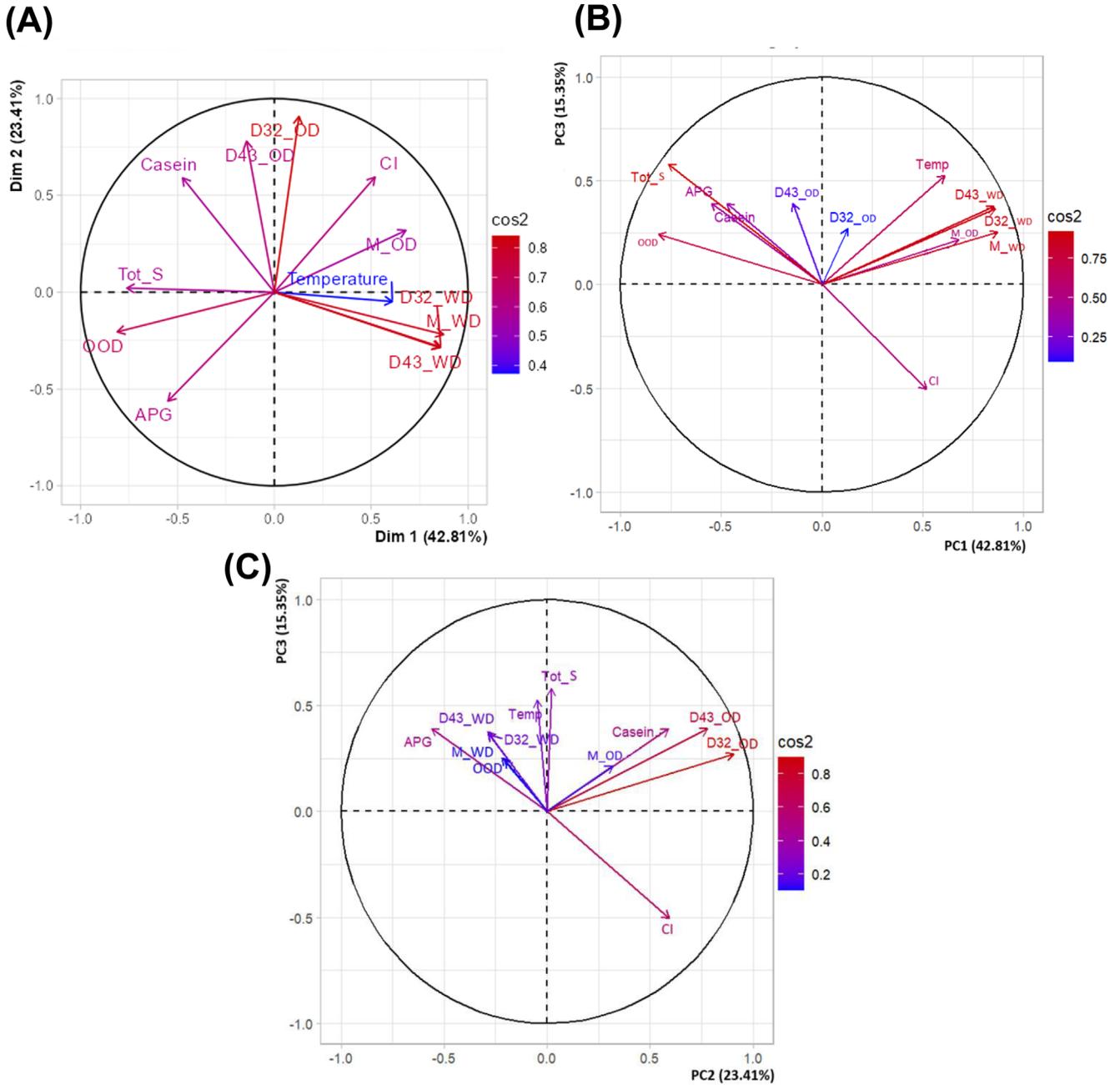


Fig. 5. Principal component analysis (PCA) plots on (A) PC1/PC2, (B) PC1/PC3 and (C) PC2/PC3. \cos^2 represents the quality of representation of a variable in a principal component.

$$Y_6(D43_WD) = 10.55 + 2.27X_1 - 2.93X_2 - 1.65X_3 + 6.48X_1^2 - 2.44X_2^2 + 1.69X_3^2, \quad (7)$$

$$Y_7(D32_WD) = 9.69 + 2.14X_1 - 2.85X_2 - 1.62X_3 + 5.81X_1^2 - 2.11X_2^2 + 1.64X_3^2, \quad (8)$$

$$Y_8(M_WD) = 7.91 + 1.34X_1 - 2.69X_2 - 1.70X_3. \quad (9)$$

The coefficients that were found to be non-significant ($p > 0.05$), were separated from the complete model to develop a reduced model (Tab. 5). The final reduced model equations are summarized as follows:

$$Y_1(OOD) = 111.52 - 15.01X_1 + 58.66X_2 + 71.28X_3, \quad (10)$$

$$Y_2(CI) = 11.28 - 9.36X_3, \quad (11)$$

$$Y_6(D43_WD) = 10.55 - 2.93X_2 + 6.48X_1^2, \quad (12)$$

Table 7. Pearson correlation coefficients of variables pairwise. OOD: onset of destabilization, CI: creaming index, D43: De Brouckere diameter, D32: Sauter diameter, M: mean diameter, OD: oil droplets, WD: water droplets in oil drops, APG: % of alkylpolyglucoside, Casein: % of Casein, Tot_S: Total % of surfactants, Temp.:temperature.

	APG	Casein	CI	D32_OD	D32_WD	D43_OD	D43_WD	M_OD	M_WD	OOD	Temp.	Tot_S
APG	1.00	-0.10	-0.76	-0.42	-0.20	-0.08	-0.19	-0.48	-0.27	0.50	-0.12	0.67
Casein	-0.10	1.00	-0.07	0.45	-0.39	0.48	-0.38	0.02	-0.48	0.40	-0.12	0.67
Id	-0.76	-0.07	1.00	0.46	0.11	0.16	0.10	0.36	0.19	-0.70	0.07	-0.62
D32_OD	-0.42	0.45	0.46	1.00	-0.05	0.89	-0.06	0.35	0.03	-0.19	0.17	0.03
D32_WD	-0.20	-0.39	0.11	-0.05	1.00	-0.20	1.00	0.53	0.94	-0.52	0.64	-0.44
D43_OD	-0.08	0.48	0.16	0.89	-0.20	1.00	-0.21	0.10	-0.10	0.01	0.00	0.29
D43_WD	-0.19	-0.38	0.10	-0.06	1.00	-0.21	1.00	0.54	0.93	-0.52	0.65	-0.42
M_OD	-0.48	0.02	0.36	0.35	0.53	0.10	0.54	1.00	0.45	-0.61	0.46	-0.34
M_WD	-0.27	-0.48	0.19	0.03	0.94	-0.10	0.93	0.45	1.00	-0.54	0.56	-0.56
OOD	0.50	0.40	-0.70	-0.19	-0.52	0.01	-0.52	-0.61	-0.54	1.00	-0.36	0.67
Temp.	-0.12	-0.12	0.07	0.17	0.64	0.00	0.65	0.46	0.56	-0.36	1.00	-0.18
Tot_S	0.67	0.67	-0.62	0.03	-0.44	0.29	-0.42	-0.34	-0.56	0.67	-0.18	1.00

Table 8. Pairwise two-sided p-values among the correlations. OOD: onset of destabilization, CI: creaming index, D43: De Brouckere diameter, D32: Sauter diameter, M: mean diameter, OD: oil droplets, WD: water droplets in oil drops, APG: % of alkylpolyglucoside, Casein: % of Casein, Tot_S: Total % of surfactants, Temp.:temperature.

	APG	Casein	CI	D32_OD	D32_WD	D43_OD	D43_WD	M_OD	M_WD	OOD	Temp.	Tot_S
APG		0.7275	0.001	0.1211	0.4745	0.7662	0.4906	0.0727	0.3343	0.059	0.668	0.006
Casein	0.728		0.811	0.0891	0.1546	0.0715	0.1681	0.9406	0.0678	0.143	0.668	0.006
CI	0.001	0.8114		0.0831	0.693	0.5594	0.7304	0.1932	0.5021	0.003	0.804	0.015
D32_OD	0.121	0.0891	0.083		0.8508	<.0001	0.8388	0.2007	0.9201	0.503	0.555	0.924
D32_WD	0.475	0.1546	0.693	0.8508		0.4688	<.0001	0.0419	<.0001	0.047	0.01	0.103
D43_OD	0.766	0.0715	0.559	<.0001	0.4688		0.4544	0.7264	0.7127	0.959	0.999	0.289
D43_WD	0.491	0.1681	0.73	0.8388	<.0001	0.4544		0.0374	<.0001	0.049	0.009	0.116
M_OD	0.073	0.9406	0.193	0.2007	0.0419	0.7264	0.0374		0.0896	0.016	0.087	0.217
M_WD	0.334	0.0678	0.502	0.9201	<.0001	0.7127	<.0001	0.0896		0.037	0.029	0.03
OOD	0.059	0.1432	0.003	0.5034	0.0466	0.9592	0.0486	0.0163	0.0367		0.186	0.007
Temp.	0.668	0.6682	0.804	0.5551	0.0099	0.9993	0.0088	0.0871	0.0286	0.186		0.521
Tot_S	0.006	0.0061	0.015	0.9241	0.1034	0.2885	0.116	0.2165	0.0301	0.007	0.521	

$$Y_7(D32_WD) = 9.69 - 2.85X_2 + 5.81X_1^2, \quad (13)$$

$$Y_8(M_WD) = 7.91 - 2.69X_2. \quad (14)$$

According to equations (10) to (14), the responses Y_1 (OOD), Y_2 (CI), and Y_8 (M_WD) follow a first order model, whereas Y_6 (D43_WD) and Y_7 (D32_WD) follow a first order model with pure quadratic terms. Y_1 (OOD) seems to be the only response that depends on all three factors. Y_2 (CI) and Y_8 (M_WD) are influenced by a single factor: the amount of surfactants. The creaming index and the average size of the inner water droplets decrease when the amount of surfactants is increased. The creaming index is influenced only by the amount of APG, while the average size of the inner water droplets is influenced only by the amount of casein. Temperature, which acts on the viscosity of the disperse phase, seems to affect only the onset of destabilization and the drop size. Indeed, the viscosity of the

oil acts as a mechanical constraint on the droplets in an emulsion. A more viscous oil generally produces larger droplets due to an increased resistance to fragmentation, which facilitates coalescence. Conversely, under similar shear forces, a less viscous oil allows the formation of smaller droplets (Brooks, 1994). In this case, not only the temperature has an impact but also the co-emulsifier (sodium caseinate). According to Huck-Iriart *et al.* (2011), the interactions between proteins (such as sodium caseinate) and triglycerides can be altered by the temperature, which can affect the stability of the emulsion.

The influence of the factors for Y_1 (OOD) and Y_6 (D43_WD) is represented by response surface plots and contours plots (Figs. 3 and 4).

For Y_6 (D43_WD), a stationary point has been calculated by RStudio software (all partial derivatives of the function are equal to zero): temperature ~ 35 °C, amount of casein = 1.8%, and amount of APG = 3.0%. On the Figure 4, we can observe that the stationary point corresponds to a saddle point which is

a local minimum of the inner droplet diameters (D43_WD). The same results were obtained for D32_WD due to the similarity of equations Y_6 and Y_7 . At these conditions, the corresponding responses are 10.8 μm for Y_6 (D43_WD), 10.1 μm for Y_7 (D32_WD), 9.7 μm for Y_8 (M_WD), 110.3 h for Y_1 (OOD) and 6.6% for Y_2 (CI).

Moreover, the surface plots show that an increasing rate of casein (up to 3.9%) enhances the stability of the emulsion and allows to further reduce the inner diameter of the droplets at 35 °C (Figs. 3 and 4). In fact, this stationary point represents the best compromise between a limited surfactant rate and a low droplet diameter.

For Y_3 (D43_OD), Y_4 (D32_OD), Y_5 (M_OD), it was not possible to define any model. This could be explained by the fact that sample LB01039 is an outlier for these responses as shown by the following box plot (Fig. 1). After removing the outlier of the CDD, the coefficients obtained are presented in Table 6. New interactions are observed for Y_1 namely between temperature and casein and between temperature and APG. Under these conditions, the size of the drops seems to depend more strongly on the temperature (p -value < 0.05) and less on the presence of casein (p -value < 0.10).

3.3 Principal component analysis

In order to assess the correlations between the different variables in greater detail, a principal component analysis (PCA) was performed to provide an overview of the data set without the outlier. The first principal component (PC1) explained 42.81% of the total variance, whereas the second principal component (PC2) explained a further 23.41%, and the third principal component (PC3) explained 15.35%. The combination of PC1, PC2 and PC3 explains 81.57% of the total variability that properly represents all the variables. Figure 5 shows the different variables on the principal components PC1/PC2, PC1/PC3 and PC2/PC3. A new variable was added to represent the total amount of surfactant in the system (Tot_S).

Figure 5 allows to assess the correlations of the variables with the principal components and between the variables. The closer the arrow is to the edge of the circle, meaning high correlation coefficients, the higher its display quality. The sum of the cosine square (\cos^2) on each axis, which is shown in colour in Figure 5, represents the quality of representation of the variables on the PCA graphs. A sum of \cos^2 close to 1 (red colour) means a high quality of representation. According to Figure 5(A), the amount of APG and the creaming index are negatively correlated, as described by the result obtained with the CCD. This result is in agreement with the high Pearson coefficient correlation of -0.76 ($p < 0.01$) (Tabs. 7 and 8). Conversely, the amount of casein is not correlated to the creaming index, as shown by a Pearson correlation coefficient of -0.07. Figure 5(B) shows that the amounts of casein and APG (Tot_S) have an impact on the onset of destabilization. The onset of destabilization is delayed with an increased amount of surfactant in the tested range, given the Pearson coefficient correlation of 0.67. We thus observe that the diameter of the inner water droplets (D43_WD, D32_WD) is not correlated with the amount of APG ($\text{PCC} < -0.3$), but is related to temperature

with a high correlation ($\text{PCC} > 0.64$ with $p < 0.01$). Indeed, the PCA shows only a linear correlation, but the CCD indicated a quadratic effect of the temperature with an optimum at 35 °C.

4 Conclusion

The present study deals with the development of new W/O/W emulsions based on sunflower oil pre-treated by cold plasma, a green technology for the pre-polymerization of vegetable oils. Experimental tests show that combining casein and APG helps to better stabilize the emulsion. Results of the principal component analysis (PCA) highlight the fact that the stability of the emulsions mainly depends on the total amount of surfactant. Indeed, increasing the surfactant content (APG + casein) up to 7% (w/w) improves the stability of the emulsion by delaying the onset of destabilization. We also observe the role of APG (SimulsolTM SL26C) to limit the creaming index (strong negative correlation) regardless the amount of casein. The temperature of emulsification has an effect on the average size of the inner water droplets. This is in accordance with the central composite design where we found that a temperature of 35 °C with a surfactant rate of 3.0% of APG and a casein rate between 1.8% and 3.9%, allows to reduce the inner water droplet diameters and confers stability to these new emulsions, over several days. The performances of these biobased emulsions could be interesting for different applications (lubricants, cosmetics, coatings ...).

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Author contribution statement

Laetitia Boisset: Formal Analysis, Investigation, Methodology, Writing-Original Draft Preparation. Brigitte Dubreuil: Formal Analysis, Writing-Review & Editing. Sophie Thiebaud-Roux: Conceptualization, Supervision. Pascale De Caro: Methodology, Writing-Original Draft Preparation, Writing-Review & Editing.

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