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Evaluation of different emulsification methods in double emulsion systems: Response surface optimization approach and emulsion properties

İkili emülsiyon sistemlerinde farklı emülsifikasyon yöntemlerinin değerlendirilmesi: Yanıt yüzey optimizasyonu yaklaşımı ve emülsiyon özellikleri

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ABSTRACT

Double emulsions have been the focus of several studies due to their advantages such as trapping dispersed phase droplets, delaying the release, masking taste-odor, and providing protection against oxidation. In this study, double emulsions (w/o/w) were prepared by different emulsifying methods including one-step, two-steps and membrane methods to assess their emulsion yield and stability. Results showed that one-step method was preferable for zeta potential and polydispersity index but similar in droplet size as in membrane method. Due to its simplicity and ease of use, one step method was further studied in an optimization study to evaluate factors affecting droplet size and polydispersity index. Under optimum conditions (oil ratio: 35.1%, emulsifier ratio: 6.9%, homogenization speed: 15000 rpm and homogenization time: 5 min), droplet size and polydispersity index were 180.7±6.6 nm and 0.13±0.01, respectively. pH of the resultant emulsion was 2.89±0.01, conductivity was 9.65±0.05 mS/cm, emulsion stability was 8.02±0.66%, and zeta potential was -50.30±1.70 mV. This study demonstrated that nano-scale emulsion and highly stable emulsions can be obtained for various applications in the food industry under optimum conditions by a single-step method.

Key Words: Droplet size, Olive oil, One-step emulsion, Polydispersity index, Zeta potential.

ÖZ

İkili emülsiyonlar, dağılmış faz damlacıklarını hapsederken salınımı geciktirme, tat ve koku maskeleme, oksidasyona karşı koruma sağlama gibi avantajları nedeniyle birçok çalışmada ilgi odağı olmuştur. Bu çalışmada, emülsiyon verimi ve stabilite açısından değerlendirmek amacıyla farklı emülsifiye yöntemleri olan tek adımlı, iki adımlı ve membran yöntemleri kullanılarak su-yağ-su (s/y/s) tipi çift emülsiyonlar hazırlanmıştır. Sonuçlar, tek adımlı yöntemin zeta potansiyeli ve polidispersite indeksi açısından daha tercih edilebilir olduğunu, damlacık boyutu açısından ise membran yöntemi ile benzer sonuçlar verdiğini göstermiştir. Basitliği ve kullanım kolaylığı nedeniyle tek adımlı yöntem, damlacık boyutu ve polidispersite indeksini etkileyen faktörlerin değerlendirildiği bir optimizasyon çalışmasında daha ayrıntılı olarak incelenmiştir. Optimum koşullar altında (yağ oranı: %35,1; emülgatör oranı: %6,9; homojenizasyon hızı: 15.000 rpm ve homojenizasyon süresi: 5 dk), damlacık boyutu ve polidispersite indeksi sırasıyla 180,7±6,6 nm ve 0,13±0,01 olarak bulunmuştur. Elde edilen emülsiyonun pH değeri 2,89±0,01, iletkenliği 9,65±0,05 mS/cm, emülsiyon stabilitesi %8,02±0,66 ve zeta potansiyeli -50,30±1,70 mV olarak belirlenmiştir.

Bu çalışma, optimum koşullar altında tek adımlı yöntemle gıda endüstrisinde çeşitli uygulamalar için nano-ölçekte ve yüksek stabiliteye sahip emülsiyonlar elde edilebileceğini göstermiştir.

Anahtar Kelimeler: Damlacık boyutu, Zeytinyağı, Tek adımlı emülsiyon, Polidispersite indeksi, Zeta potansiyeli.

Introduction

Emulsion is formed by dispersion of two insoluble liquids as small droplets in one another. As food systems are complex matrices of various ingredients that do not always mix well, oil-water and/or air-water interfaces are common and can be stabilized using suitable emulsifying or surface stabilizing agents (Hu et al., 2017). Double emulsions are complex dispersion systems characterized by low thermodynamic stability as they are 'emulsions of emulsions'. Double emulsions can be obtained in two forms; water-in-oil-in-water droplets (W/O/W) or oil-in-water-in oil droplets (O/W/O), in where they can be used to encapsulate active ingredients in numerous applications, including drug delivery, foods, cosmetics, chemical separations, and synthesis of microspheres and microcapsules (Chu et al., 2007). Potentially, W/O/W emulsions have some advantages over traditional O/W emulsions, such as delivery systems for bioactive lipids as well as encapsulation, protection and release of hydrophilic components as they have the ability to retain and protect those substances and control their release from one phase to another (Jiménez-Colmenero, 2013). Double emulsions have been utilized in various food systems. For example, they have been applied in imitation creams, reduced-fat mayonnaise, and in the production of spreadable products using W/O/W emulsions containing butter flavor. In addition, multiple emulsions have been employed as fat replacers in meat emulsions, high-sugar fillings for confectionery, substitutes for milk fat (in milk and cheese), nutrient-loaded capsules (such as sodium ascorbate in milk, $CaCl_2$ in soybean products, and $MgCl_2$ in tofu), components designed to modify fatty acid composition (in spreads and meat/dairy emulsions), and structures for the fortification of dairy products with vitamins. More ambitiously, food-related applications of multiple emulsions have also aimed to prolong the release of sweeteners in chewing gum, produce edible films with increased water permeability, and provide long-term protection to specific sensitive compounds (such as curcumin in beverages, natural colorants in confectionery, and bioactives in ice cream and fruit-based products) (Muschiolik & Dickinson, 2017). Gracher-Teixeira et al., 2024 demonstrated the potential of double emulsions to preserve natural colorants, enhance color stability, and their suitability for food industry applications. Alouk et al., 2023 demonstrated that double emulsion systems are effective for the stability, bioavailability, and controlled release of natural pigments.

There are a few methods in use for emulsions including one step, two steps and membrane methods, just to mention the most common. The most widely used method for multiple emulsion production is two-step emulsion method. To prepare this emulsion, the inner aqueous phase (W1) is dispersed in oil phase containing a lipophilic emulsifier at first, then the first emulsion obtained is dispersed into outer aqueous phase (W2) containing a hydrophilic emulsifier this time. Alternatively, a microfluidic approach has been developed with various flow focusing techniques (Kim et al., 2018). Recently, a one-step emulsification process has been reported for W1/O/W2 emulsions in which both lipophilic and hydrophilic surfactants are added to the oil phase, simultaneously (Pradhan and Rousseau, 2012). Wu et al. (2019) demonstrated that single-step emulsification reduces processing time while maintaining droplet integrity and the encapsulation efficiency of biologically active components. Although the physical and structural properties of double emulsions (e.g., droplet size, zeta potential, and polydispersity index) are widely used as stability indicators, there are very few studies that directly compare these parameters under different production methods (Kupikowska-Stobba et al., 2024; Tadros, 2013).

Pasban et al. (2023) reported that high-pressure homogenization reduced droplet size and increased zeta potential, thereby improving emulsion stability. Leister (2020) and Karbstein emphasized that larger droplet sizes increase the cream rate and thus negatively affect stability in double emulsions. Therefore, research aimed at determining which production method provides the most stable and reproducible systems, particularly when using food-grade oils such as olive oil, represents a significant gap in the literature. Comparative evaluations of different methods used in the production of multiple emulsions in the literature are limited, and systematic studies aimed at optimizing a specific production method are insufficient. Furthermore, these methods have never been compared in terms of emulsion yield and stability as the relevant literature mostly focused on the emulsions of different edible or inedible ingredients and their incorporation into food, cosmetics and drugs (Yildirim et al., 2017). In addition, emulsion parameters such as temperature (°C), homogenization rate (rpm), homogenization duration (min), concentration of the emulsifying agent, type of the emulsifying agent and the ratio of oil and water phases have not been thoroughly studied for their effects on the stability and quality of the emulsions obtained. Therefore, this study was designed to compare double emulsions of olive oil produced by three different methods to assess them in terms of droplet size, zeta potential and polydispersity index as indicators of emulsion stability. This study addresses a significant gap in the literature by comparing the physicochemical, structural, and stability properties of multiple emulsions obtained using three different production methods and selecting the most suitable method for optimization. Luo and Wei (2023) systematically examined the effects of production techniques, emulsion components, and process conditions on stability; they emphasized that there is still no clear consensus on which methods yield more reproducible and functional results. The determination of the optimization parameters of the selected method increases the repeatability of the production process and contributes to the development of controlled and stable multiple emulsion formulations suitable for industrial applications. After the preliminary studies, one-step method was further evaluated in an optimization study to determine optimum process conditions for double emulsion of olive oil.

Materials and Methods

Materials

Virgin olive oil (91%, produce of 12.2022) was purchased from a local market. Span 80 and tween 20 were purchased from Sigma Aldrich (St. Louis, USA). All other chemicals were of analytical grade and purchased from Sigma Aldrich (St. Louis, USA).

Methods

Production of emulsions

One step emulsion was obtained by slight modifications to the method given by Wang et al. (2017). Both hydrophilic and lipophilic emulsifiers were added to the oil at the same time by mixing 2 g of oil + 0.16 g of tween 20 + 0.04 g of span 80 and this mix was first stirred using a magnetic stirrer (Heidolph MR Hei-Tec, USA) at 800 rpm for 15 min. Then, 8 g of distilled water + 0.1 g of NaCl was added and mixed again at 800 rpm for 30 min. After 10 min rest, the mixture was homogenized using an homogenizer (T18 Digital Ultra Turrax®, IKA-Werke GmbH & Co. KG, Staufen, Germany) at 10000 rpm for 5 min (Figure 1, left).

For two steps emulsion; W/O emulsion was first obtained and added to the water phase (Figure 1, right) to obtain W/O/W double emulsion. For this purpose, first 2 g of oil + 0.04 g of span 80 was stirred at 375 rpm for 5 min. Then, 1 g of distilled water was added and stirred for 5 min as before. Then, the mixture was homogenized at 10000 rpm for 10 min. For the continuous water phase, 7 g of distilled water + 0.14 g of tween 20 + 0.1 g of NaCl were stirred at 375 rpm for 5 min. Lastly,

dispersed oil phase was added and stirred as before and then homogenized at 10000 rpm for 5 min (Poyato et al., 2013).

Preliminary steps were the same in both two steps and membrane methods except that a syringe (2.5 mL, transparent plastic syringe) was used in membrane method when adding the dispersed phase to the continuous phase. That is, the water phase was added to the oil phase using a syringe for formation of the W/O primary emulsion and then, this primary emulsion was added to the water phase with a syringe as before for formation of the W/O/W double emulsion. Photographs of emulsions produced with these methods are given in Figure 2.

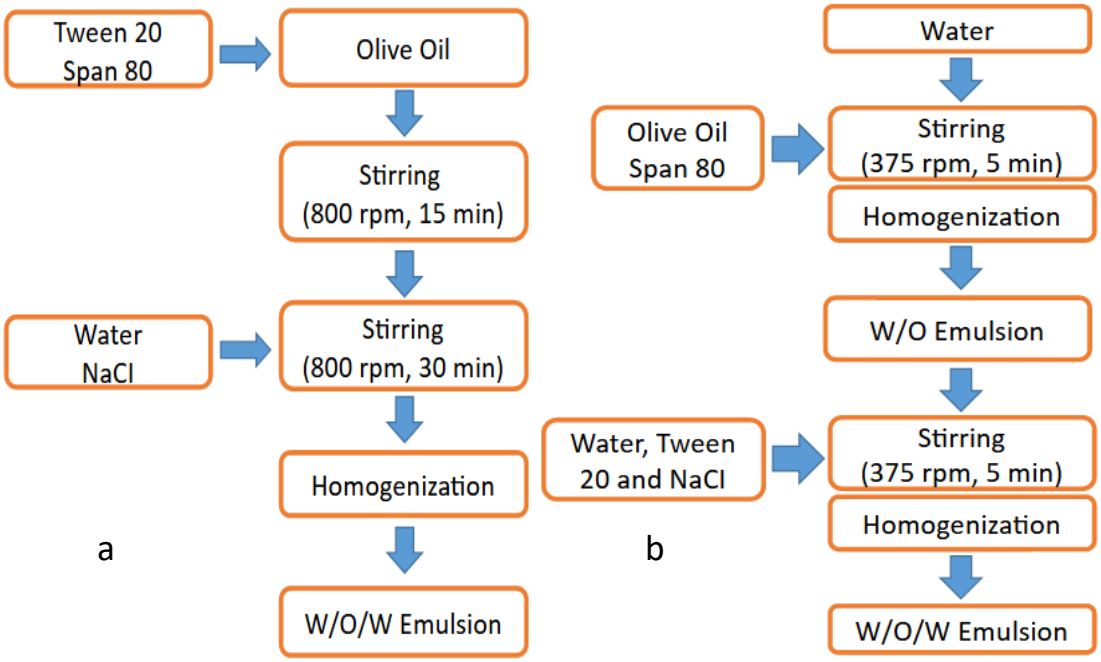

Figure 1. Scheme of process flow for one step emulsion (a), two steps emulsion (b).

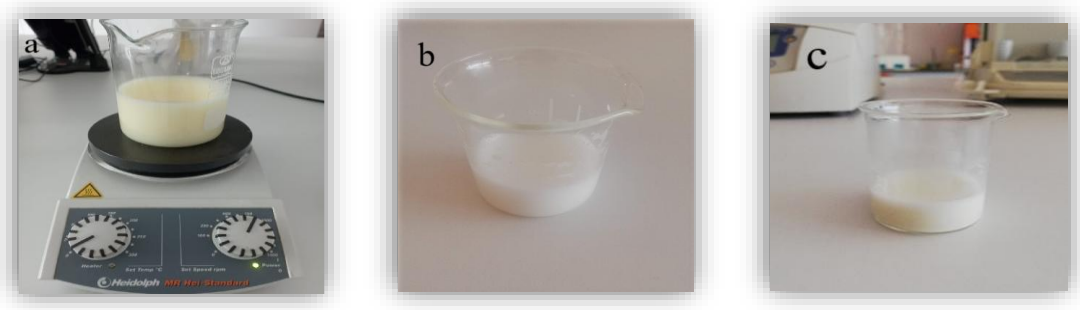

Figure 2. Emulsions produced by one-step emulsion (a), membrane emulsion (b) and two-step emulsion (c) methods.

Assessment of the emulsions and the experimental plan for optimization

Double emulsions of olive oil obtained by one step, two steps and membrane methods were compared by their droplet size, zeta potential and polydispersity index. One step method was superior in terms of zeta potential and polydispersity index indicating higher emulsion stability although droplet size was similar in one step and membrane methods. Despite that, one step method was further studied in optimization due to its simplicity and ease of use. Therefore, an optimization study was designed using JMP statistics software (SAS, NC, USA). Zeta potential was not selected as one of the responses at this stage. Because droplet size provides information about emulsion stability. Therefore, polydispersity index was selected as one of the responses instead of zeta potential. Response surface method and central composite design for 4-factors at 3-levels with 3 central points and total

of 27 experimental runs were used. Independent variables were the ratio of oil:water phases (20:80, 30:70 and 40:60), emulsifier concentration (4, 6 and 8%), homogenization rate (7500, 10000 and 15000 rpm) and homogenization duration (5, 7.5 and 10 min) while dependent variables were droplet size and polydispersity index, both to be minimized.

Acid value, pH and conductivity

The acid value was measured according to the AOCS (Cd 3a-63), (AOCS, 1989). pH and conductivity of the oil sample and the emulsions obtained were determined by a multi-meter (SG7, Mettler Toledo, OH, USA) at ambient temperature. Approximately 20 mL of the sample was transferred to a beaker and the measurement was carried out as reported by Abdolmaleki et al. (Abdolmaleki et al., 2016).

Viscosity

Viscosity measurement was carried out as reported by Moayedzadeh et al. (2018). Viscosity of the samples was measured at 25°C and 40 rpm using a Brookfield DVIII Ultra rheometer (Brookfield, USA) and small sample adapter.

Color

Color measurement was carried out using a colorimeter (CSM4, PCE Instruments GmbH, Germany). Samples were placed in a petri dish and then, L, a, and b values were read by the colorimeter. Whiteness index (WI) was also calculated by the formula given below (Liu et al., 2019).

$$WI = 100 - \sqrt{(100 - L)^2 + a^2 + b^2}$$

Zeta potential, droplet size and polydispersity index

Zeta potential, droplet size and polydispersity index were measured using a zetasizer (Nano ZPS, Malvern Instruments, England). Samples were diluted with pure water before analysis to avoid multiple scattering. Samples were placed in a disposable folded capillary cell with electrodes. An average of 30 measurements were made for each sample at 25°C (Moayedzadeh et al., 2018)

Emulsion stability

Emulsion stability (ES) was determined by the method reported by Klaypradit and Huang (2008) with slight modifications. Emulsion samples (8 mL) were placed in a test tube and stored at ambient temperature for a month. Then, serum height (SH) accumulated at the top was measured and compared with the initial emulsion height (EH) and ES was calculated by the formula below.

ES = (SH / EH) × 100

Fourier transform infrared (FTIR) spectroscopy

FTIR spectra of the samples were obtained with 32 scans in the range of 4000–650 cm^{-1} with a resolution of 4 cm^{-1} (Nicoleti S10, Thermo Scientific, USA) (Albano et al., 2020).

Optical microscopy

A drop of freshly prepared emulsion was diluted 10 folds (10% emulsion in distilled water, w/w), then placed on a glass slide and gently covered with a cover slip. Images were obtained using an optical microscope (Eclipse E600, Nikon, Japan) at a magnification of 400× and 1000× (Albano et al., 2020).

Statistical analysis

Data was evaluated using JMP 8.0 (SAS, NC, USA) software. Optimization of the process parameters were achieved using prediction profiler by maximizing the desirability. The data obtained in the study was evaluated with ANOVA and Tukey-Kramer tests and significant differences were reported within 95% probability limits.

Results and Discussion

Some quality characteristics of olive oil

Some of the quality features of olive oil are given in Table 1. Initial acid value was 3.10±0.28 mg KOH/g oil, lower than 6.08 mg KOH/g reported by Abderrazzaq and Hijazi (2012). No conductivity was in olive oil while its viscosity was 11.25±0.00 cP. L (brightness) value was 59.37±0.53 while a

(redness-greenness) value was -0.38±0.05 and b (yellowness-blueness) value was 42.45±1.58. Whiteness index was 41.23±1.29 (Table 1).

Double emulsions obtained by different methods

Three different procedures were evaluated for production of olive oil emulsions and efficiency of these procedures were assessed based on droplet size, zeta potential and polydispersity index (Table 2). Stability of an emulsion depends on both its content and droplet size as smaller droplet size leads to lasting stability in emulsions. Zeta potential is an indicator of the potential stability of an emulsion as zeta potential lower than -30 or higher than +30 mV is considered stable (Larsson et al., 2012). Based on the results, the smallest droplet size and the lowest zeta potential was for one-step emulsion method. One-step emulsion method was also practical and fast. Therefore, one-step emulsion method was further studied for optimization of process parameters.

Table 1. Some properties of olive oil.

Parameters	Value
Acid value (mg KOH g^{-1} oil)	3.10±0.28
Conductivity (mS cm^{-1})	0.00±0.00
Viscosity (cP)	11.25±0.00
L	59.37±0.53
a	-0.38±0.05
b	42.45±1.58
WI	41.23±1.29

Optimization of process parameters

Experimental design and results of the optimization study are given in Table 3. Effect of homogenization speed on droplet size and polydispersity index was significant ($P<0.05$). As homogenization speed increased, droplet size and polydispersity index decreased. Effect of homogenization duration was non-significant for both dependent variables. Increasing emulsifier concentration decreased droplet size and polydispersity index. At higher homogenization rates, higher energy density is applied to the solution, which directly reduces droplet size of the emulsion (Mulia et al., 2019). Similarly, higher oil ratio caused lower values of both dependent variables up to a certain level, but the upper end level of oil ratio led to larger droplet size as expected. As the oil concentration increases, the emulsifier becomes insufficient to stabilize all droplets, leading to enhanced coalescence. (Dapčević Hadnađev et al., 2013).

Table 2. Droplet size and zeta potential of the emulsions.

	Droplet size (nm)	Zeta potential (mV)	Polydispersity index
One step method	247.9±8.0^{B}	-41.05±0.76^{C}	0.168±0.01^{B}
Two steps method	337.5±35.3^{A}	-35.77±1.40^{B}	0.224±0.02^{A}
Membrane method	248.2±8.1^{B}	-27.74±0.63^{A}	0.170±0.00^{B}

The regression equations obtained were as given below. Equation 3.1 is for droplet size (DS) and Equation 3.2 is for polydispersity index (PI), in which the oil ratio was given as OR and the homogenization speed was given as HS. Response surface figures of both dependent variables are given in Figure 3.

$$DS=2.204+(0.147\times OR)-(0.256\times HS)+(0.236\times OR^2)+(0.122\times HS^2) \quad (3.1)$$

$$PI=0.200-(0.077\times HS)-(0.045\times OR\times HS)+(0.060\times OR^2)+(0.051\times HS^2) \quad (3.2)$$

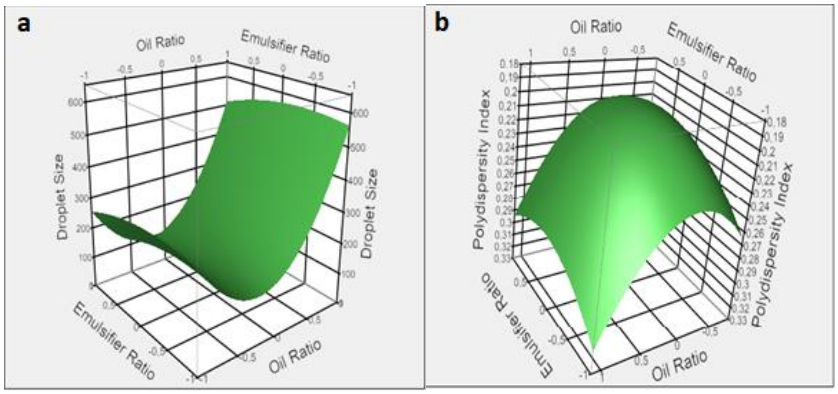

Figure 3. Response surface graphics for droplet size (a) and polydispersity index (b).

Optimum levels of the independent variables were 0.51, 0.45, 1 and -1 for oil ratio, emulsifier ratio, homogenization speed and homogenization duration, respectively (Table 4). Corresponding values of these variables were 35.1%, 6.9%, 15000 rpm and 5 min, respectively. Estimated values of dependent variables under optimum conditions were 177.81±1.53 nm (R^2=0.94) for droplet size and 0.14±0.08 (R^2=0.88) for polydispersity index (Table 5). Corresponding experimental values were 180.7±6.6 nm and 0.13±0.01. These values show a reasonably good match between estimated and experimental results, which indicates success of the model.

Table 3. Experimental results for optimization study of one step emulsion.

Sample No	Oil:Water concentration (%)	Emulsifier concentration (%)	Homogenization speed (rpm)	Homogenization duration (min)	Droplet size (nm)	Polydispersity index
1	30:70	6	15000	7.5	138.6	0.212
2	20:80	8	15000	10	126.5	0.258
3	30:70	6	10000	7.5	159.1	0.181
4	20:80	4	15000	5	133.2	0.262
5	30:70	6	7500	7.5	430	0.29
6	20:80	8	7500	5	511.77	0.322
7	30:70	4	10000	7.5	138.9	0.284
8	20:80	8	15000	5	132.5	0.241
9	30:70	6	10000	7.5	164.7	0.194
10	20:80	4	15000	10	162.3	0.277
11	40:60	8	15000	5	296.73	0.214
12	30:70	8	10000	7.5	149.2	0.178
13	30:70	6	10000	7.5	152.4	0.178
14	20:80	6	10000	7.5	131.9	0.28
15	30:70	6	10000	5	180.7	0.128
16	20:80	4	7500	5	641	0.378
17	40:60	4	15000	5	336.6	0.224
18	40:60	8	15000	10	269.8	0.186
19	20:80	4	7500	10	456.17	0.308
20	40:60	4	7500	5	902.83	0.518
21	40:60	8	7500	10	817.1	0.498
22	40:60	8	7500	5	653.13	0.313
23	40:60	6	10000	7.5	762.47	0.24
24	30:70	6	10000	10	136.1	0.26
25	40:60	4	15000	10	257.8	0.179
26	40:60	4	7500	10	763.67	0.403
27	20:80	8	7500	10	557.17	0.322

Some characteristics of the double emulsion

Double emulsion obtained under optimum conditions was analyzed for its acid value, pH, conductivity, viscosity, color, zeta potential, polydispersity index, droplet size and emulsion stability.

Table 4. Optimum double emulsion conditions.

Variables	Level	Value
Oil:Water concentration	0.51	35.1:64.9%
Emulsifier concentration	0.45	6.9%
Homogenization speed	1	15000 rpm
Homogenization duration	-1	5 min

Table 5. Predictive and experimental data under optimum conditions.

Droplet Size (nm)		Polydispersity index	
Predictive	Experimental	Predictive	Experimental
177.81±1.53 (R^2=0.94)	180.7±6.6	0.14±0.08 (R^2=0.88)	0.13±0.01

Acid value was 1.50±0.20 mg KOH/g oil and pH was 2.89±0.01 (Table 6). Khor et al. (2014) reported pH values of four different commercial emulsions of olive oil as 2.52, 2.63, 2.85 and 3.45; which is in good agreement with the present study. Low pH values can be attributed to the effect of the ionic structure of protonated components in the internal phase and the emulsifiers used. Low pH values can increase emulsion stability by strengthening the surface charge of droplets (Sarkar and Dickinson, 2020). Moulai-Mostefa and Bounmenir (2010) studied the conductivity of multiple emulsions obtained by one-step method at different oil and emulsifier ratios and reported conductivity values between 5.15 and 8.8 mS cm^{-1}, which was slightly lower than the value (9.65±0.05 mS cm^{-1}) obtained in the present study. Since salt is used in emulsion production, conductivity was high compared to oil itself. Harnsilawat et al. (2006) reported that pH and ionic strength (0–250 mM NaCl range) significantly affected droplet charge, droplet size, and cream stability; an increase in NaCl concentration could alter droplet interactions and thus emulsion stability by increasing conductivity and causing changes in zeta potential. Viscosity was 2.50±0.00 cP, which was relatively low due to smaller droplet size. Viscosity of oil appears to decrease with incorporation of oil into double emulsion. Viscosity is higher for emulsions with larger droplet size (Khadem and Sheibat-Othman, 2019). Viscosity in emulsions is generally dependent on droplet size and distribution. As droplet diameter decreases, the flow resistance of the system decreases, which causes viscosity to decrease (McClements, 2004). The low viscosity value obtained in this study can be explained by the small droplet diameters and the inclusion of the oil phase in the double emulsion structure. L represents the lightness of the sample, while a and b give the color coordinates. L of double emulsion was 97.93±0.04 while a was -7.59±0.29, b was 10.95±0.18 and whiteness index was 86.52±0.31. It is reported that smaller droplet size leads to increased brightness (Chantrapornchai et al., 1998). In general, these results indicate that the physicochemical properties of the emulsion—particularly the pH, conductivity, viscosity, and color parameters—are interrelated. Low pH and high conductivity enhance the electrostatic stability of the system, while small droplet size both reduces viscosity and increases color clarity.

Zeta potential, droplet size and polydispersity index of double emulsion are given in Table 6. Zeta potential was -50.30±1.70 mV. Zeta potential contributes to stability of double emulsions as strong electrostatic repulsion. Similar results were previously reported by Kim et al. (2016) for W/O/W emulsions produced from soybean oil. Droplet size was 180.7±6.6 nm and polydispersity index was 0.128±0.006, which show high stability of the emulsion obtained. Polydispersity index (PDI) is dimensionless and scaled so that values less than 0.05 appear primarily in highly monodisperse standards. PDI values greater than 0.7 indicate a very broad particle size distribution. Numerical value of PDI ranges from 0 (for a perfectly uniform sample) to 1. Values lower than 0.2 are considered acceptable in practice for polymer-based nanoparticle materials (Danaei et al., 2018). A droplet size of approximately 180 nm and a PDI value of ~0.128 indicate that the system is highly monodisperse and has a narrow droplet distribution. This narrow distribution indicates a low tendency for migration, coalescence, or separation at different speeds between droplets. Emulsion stability was 8.02±0.66%, which was in good agreement with previously reported values by Li et al. (2014). Emulsion stability between 0 and 20% indicates lower serum separation and higher emulsion stability. An emulsion stability of ~8% indicates a low level of phase separation.

Table 6. Properties of one-step olive oil double emulsion

Parameters	W/O/W
pH	2.89±0.01
Acid value (mg KOH g^{-1} oil)	1.50±0.20
Conductivity (mS cm^{-1})	9.65±0.05
Viscosity (cP)	2.50±0.00
L	97.93±0.04
a	-7.59±0.29
b	10.95±0.18
WI	86.52±0.31
Zeta potantial (mV)	-50.30±1.70
Droplet size (nm)	180.7±6.6
Polydispersity index	0.128±0.006
Emulsion stability (%)	8.02±0.66

Optical microscopy and FTIR

Microscopy images are given in Figure 4 and as seen droplets were distributed quite homogeneously. Furthermore, structure of W/O/W double emulsion was clearly visible in the images, indicating successful formation of double emulsion. In the images, the inner and outer phase boundaries of the W/O/W double emulsion were clearly observed, and it was seen that the inner water droplets were evenly distributed within the oil phase and that there were minimal signs of coalescence or fusion. These findings indicate that the double emulsion was successfully formed and that droplet integrity was maintained.

FTIR scans of olive oil and double emulsion are given in Figure 5. Peak seen at 3347 cm^{-1} belongs to the O-H stretch. Peak observed at 1637 cm^{-1} was associated with symmetric and asymmetric stretching vibrations of -COOH groups. Peak seen at 1091 cm^{-1} was due to the C-O tension. Peaks at 2922 and 2853 cm^{-1} seen in olive oil were due to the asymmetric and symmetric stretching vibration of the methylene ($-CH_2$) group. Peak at 1744 cm^{-1} was due to the ester carbonyl functional group of triglycerides. Peaks were due to bending vibrations of CH_2 and CH_3 aliphatic groups at 1461cm^{-1}, bending vibrations of CH_2 and CH_3 aliphatic groups at 1376 cm^{-1}, from C-O stretching at 1236 and 1160 cm^{-1}, from C-O ester group stretching vibrations at 1117 and 1095 cm^{-1}, from shaking vibration of methylene ($-CH_2$) and overlapping of out-of-plane vibration of cis-disubstituted olefins at 721 cm^{-1} (Rohman and Che-Man, 2010). The peaks seen in olive oil were also seen as small signals in the emulsion. The observation of these peaks as small signals in the emulsion indicates that the oil phase is preserved within the emulsion and that structural integrity is maintained. Unlike olive oil, peak seen at 1637.88 and 3347.14 cm^{-1} was due to span 80 and tween 20, respectively (Khan et al., 2015). These peaks indicate that emulsifiers settle at the oil-water interface due to their surface-active properties and increase droplet stability by regulating hydrogen bonds. Furthermore, the preservation of ester carbonyl and aliphatic groups in olive oil confirms that the lipid structure is not disrupted during emulsion preparation. These results demonstrate that the prepared W/O/W double emulsion is suitable in terms of both structure and function.

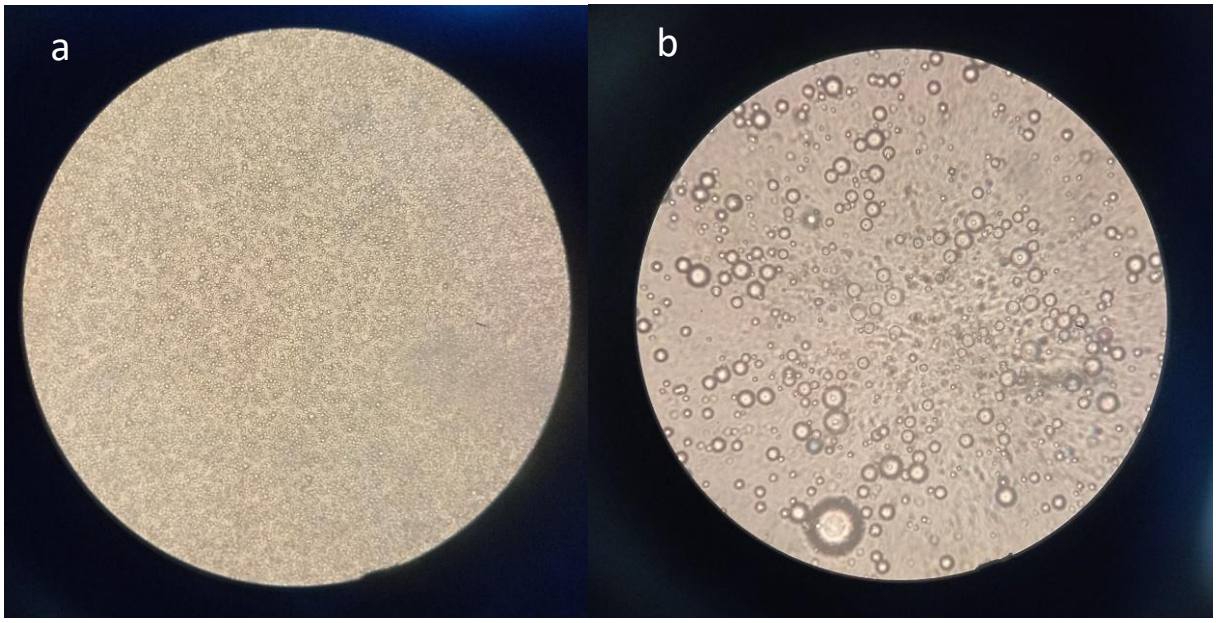

Figure 4. Microscope images of double emulsion obtained by one step method under optimum conditions (a: 400×, b: 1000×).

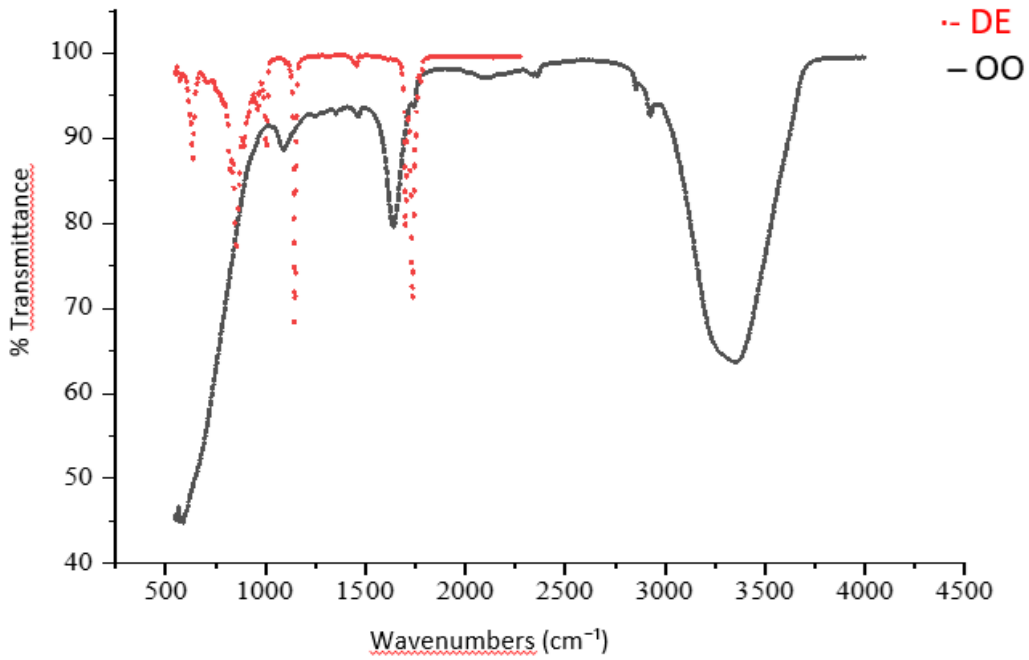

Figure 5. FTIR scan of olive oil (OO) and its double emulsion (DE) obtained by one step method under optimum conditions (b).

Conclusions

In this study, olive oil was used for production of multiple emulsions, which were evaluated for selection of the best emulsion method and for optimization of process parameters. Preliminary studies showed that one-step emulsions led to smaller droplet size and consequently stronger stability. Therefore, optimization study was carried out with one-step emulsions and optimum conditions based on the experimental results were as follows: 35.1% of oil ratio, 6.9% of emulsifier ratio, 15000 rpm of homogenization speed and 5 min of homogenization duration. Experimental and estimated values of the dependent variables were 180.7+6.6 and 177.81+1.53 nm for droplet size, and 0.13+0.01 and 0.14+0.08 for the polydispersity index, respectively. It was determined that the emulsions produced under optimum conditions had relatively smaller droplet size, low polydispersity index, low viscosity values and

lasting emulsion stability. It is concluded that one step method was not only superior in terms of droplet size and emulsion stability but also fast and practical compared to the other methods studied for emulsion production.

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Author Contributions

Gülistan OKUTAN: Data curation, Formal analysis, Investigation, Methodology, Resources, Validation, Visualization, Writing – original draft, Writing – review and editing

Gökhan BORAN: Conceptualization, Data curation, Formal analysis, Funding acquisition, Project administration, Resources, Supervision, Writing – original draft, Writing – review and editing

Competing Interests

There is no conflict of interest among the authors of the article

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