Application of Oleogel and Conventional Fats for Ultrasound-assisted Obtaining of Vegan Creams

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Abstract: The purpose of this study was to determine the impact of the fat system type (milk fat - MF, palm oil - PO or oleogel - OG, i.e. RO-LO – rapeseed oil and linseed oil mixture structured by candelilla wax) on the properties of soy creams, in comparison with dairy cream. The MF exhibited the most increase of acid value (2.5-fold), and the RO-LO – increase of peroxide value (3-fold), after 30 days of storage at 20°C. The PO was the most oxidative stable. The OG presented the slightest oxidative changes, the highest slip melting point (39°C) and centrifugal stability (99.6%). The pH and total acidity values of soy creams were similar to soy drink. All creams exhibited unimodal distribution of dispersed particles. The average particle size and dispersity indexes of these emulsions were in range of 1.74-1.80 µm and 0.93-1.16, respectively. The creams with MF or OG exhibited a greater viscosity than sweet dairy cream – 1.66 10⁻⁵ nm², and a higher degree of shear-thinning. The accelerated creaming phenomenon (flotation of lipids molecules) occurred during centrifugation. The cream with PO had the lowest resistance to centrifugal force (instability index – 0.052). The possibility to obtain a stable vegan soy creams containing oleogel (as replacer of conventional fats) has been demonstrated.

Key words: oleogel, candelilla wax, palm oil, milk fat, vegan cream, peroxide value, microrheology, STEP-technology, optical methods

1 Introduction

Solid and semi-solid fats are widely used by the food industry. The high utility value of these fats mainly is a consequence of the high content of saturated fatty acids (SFAs). Moreover, despite the advances in fat processing/modification technology, foods still contain trans fatty acids (TFAs), mainly due to hardening by partial hydrogenation¹,². In general, the SFAs contribute to increasing the risk of cardiovascular disease, and the dietary guidelines recommend limited consumption of products with high SFAs content, regardless of origin. The total intake of SFAs should be less than 10% of total energy per day³,⁴. SFAs ought should be replaced in a diet with unsaturated fatty acids⁵. Palm oil, which is widespread in the food industry, is a natural plant-based alternative to milk fat. Such replacement is not the solution to the SFAs problem, although it brings economic and technological benefits. In recent years, the cultivation of oil palm trees has caused much controversy related to deforestation, climate change, and socio-economic instability⁶. Currently, the newest and least known method, that allows to obtain a compromise between the composition and utility value of fats, is the oleogelation method. It is possible to obtain a solid/semi-solid fat system (oleogel) at room temperature, with a high content of unsaturated fatty acids (without changing the chemical composition of the oil), by using a small amount of a structuring agent (a few wt %), e.g. waxes, monoacylglycerols, ethyl cellulose¹,⁷. Edible oleogels are a future-proof solution that can reduce the consumption of TFAs and SFAs (delivering more essential unsaturated fatty acids) as well as minimalize or eliminate some technological limitations in food production⁸⁻¹⁰.

Emulsions are systems containing a fat and they are very good matrices for model laboratory tests as well as for food design on an industrial scale. Examples of such products are dairy products such as yogurts, drinks, sour cream or...
sweet cream. It is worth noting that the environmental concerns and health problems scale (allergies to milk components, lactose intolerance, or consumption of cholesterol related to dairy production and their consumption is increasing. This contributed to the enhancement of demand for alternative products. The market of milk-like plant drinks obtained from legumes, nuts, or grains is gradually developing\textsuperscript{(12)}. The most common is a soy drink, containing proteins in an amount similar to that of cow’s milk (in the range of 3-4 wt%) with slight differences in protein digestibility and amino acid profile\textsuperscript{(31)}. In addition to high nutritional value, the soybean proteins are well-known effective emulsifiers in food models\textsuperscript{(44)}.

In the literature on the subject, considerations are made on substitutes for solid fats with a high content of SFAs\textsuperscript{(15-18)} or alternatives to animal milk\textsuperscript{(19-22)}. There is a lack of research on the design of vegan products based on O/W emulsions with structured lipids (oleogels). A valuable contribution can also be taking advantage of natural emulsifier (soy protein) presence in the aqueous phase, as a stabilizer of the system, without incorporation of additional stabilizing substance to the formulations.

The purpose of the study was the attempt to obtain a vegan cream with the use of soy drink as the aqueous phase and oleogel, palm oil, or milk fat as the fat phase, as well as to evaluate the influence of the type of lipid on the physical stability of the obtained soy creams, compared to the dairy cream.

2 Materials and Methods

2.1 Materials

The following fat raw materials were investigated: milk fat – MF (clarified butter, SM Mlekovita, Poland; 99.8 wt% fat, 0.1 wt% protein), refined palm oil – PO (Kruszwica SA, Poland), refined rapeseed oil – RO (Kruszwica SA, Poland), refined linseed oil – LO (Goccia D’Oro, Italy), candellilla wax (Koster Keunen, The Netherlands; AV approx. 18 mg KOH/g, melting point approx. 70°C) and vegan creams obtained with these fats (MFS, POS, OGS). The aqueous phase of creams was an ecologic soy drink (GoBio, Austria; 2.2 wt% fat, 3.8 wt% protein). Dairy cream (Laciata UHT, Mlekpol, Poland; 30 wt% fat, 2.2 wt% protein) was considered as a comparative sample.

2.2 Preparation of oleogel

Rapeseed oil and linseed oil were mixed in a weight ratio of 1:1 (97 wt%). The candellilla wax at the level 3 wt% was incorporated into the mixture, which then was heated in a water bath (MLL 547, AJL Electronics, Poland) at 80°C for 10 min and sonicated for 15 s (26 kHz, 72 W, 100% amplitude, 15 mm sonotrode immersion) using an ultrasonic homogenizer UP200St equipped with a titanium S26d7 sonotrode (Hielscher Ultrasonics GmbH, Germany). Such prepared mixture was left in the thermostatic cabinet (20°C) for 24 h to form the structure.

2.3 Analyses of fat systems

Fatty acids composition of the lipids was determined using GC-FID system (TRACE 1300, Thermo Fisher Scientific, Waltham, USA) equipped with a BPX 70 capillary column (60 m × 0.22 mm ID × 0.25 μm). The fatty acids methyl esters (FAME) were prepared and analyzed according to ISO 12966-1:2015-01 standard\textsuperscript{(45)} with some modifications. 0.1 g of lipid was transferred into a glass tube, 2 mL of n-hexane was added, then mixed using a vortex shaker (lab dancer, VWR International Sp. z o.o., Poland) for 30 s and kept in a water bath (AJL Electronic, Poland) at 65°C for 10 seconds, until complete dissolution. 1 mL of 0.5 M KOH solution prepared in methyl alcohol was added to the tube and mixed for 30 s using a vortex. 4 mL of distilled water was poured into the tube, mixed vigorously and left for 40 min. 500 μL of clarified top layer and 500 μL of n-hexane were transferred to GC vials and placed in an auto sampler. The following parameters of analysis were set: inlet temperature: 240°C; injection volume: 0.8 μL; split ratio: 120:1; carrier gas: helium; pressure flow: 0.75 mL/min; oven temperature: 80°C for 2 min; increasing to 230°C at a rate of 2.5°C/min and holding at that temperature for 6 min; detector temperature: 280°C; detector gases: hydrogen: 40 mL/min, air: 450 mL/min, helium: 30 mL/min. The authentic standards (Food Industry FAME Mix, RESTECK, U.S.) were used for identification of fatty acids. The analyses was performed in triplicate.

The acidity value (AV) and peroxide value (PV) of lipids were determined according to the normalized standards\textsuperscript{(23, 24)}. Analyzes were performed on fresh samples (after solidification) and after 30 days of storage at 20°C (thermostatic cabinet ST 2/2+, POL-EKO APARATURA, Poland). The slip melting point (SMP) of fats was determined by the open capillary method in accordance with the methodology provided in ISO 6321-2004\textsuperscript{(25)} and the scientific work by Zbikowska et al.\textsuperscript{(26)}. The mean of five parallel determinations was taken as the final result. Centrifugal stability of fats was also indicated. For this purpose, approx. 3 g of fat was centrifuged in a centrifuge MPW-352 (MPW Med. Instruments, Poland) at 4000 r/min for 15 min. If the lipid liquid phase was separated from oleogel or MF, it was relatively unstable. After centrifugation, the tubes were inverted for 30 min to remove the released oil. The centrifugal stability of fats was calculated based on the following slight modified equation\textsuperscript{(16, 27)}:

$$CS = \frac{m_0 - m_i}{m_0 - m_f} \cdot 100\%$$

where: CS – centrifugal stability (%), m<sub>f</sub> – weight of fat and test tube after centrifugation without the released oil (g), m<sub>i</sub> – weight of fat and test tube before centrifugation (g), m<sub>0</sub> – initial weight of the testing tube (g).
m – weight of the test tube (g).

### 2.4 Preparation of creams

The fat phase (MF, PO, or OG) and the aqueous phase (soy drink) were heated to 55°C and immediately homogenized using a UP200S ultrasonic homogenizer equipped with a titanium S26d7 sonotrode (Hielscher Ultraso
cins GmbH, Germany), using the parameters: sonication time – 1 min, amplitude – 80%, pulse – 100%, power – 48 ± 2 W, supplied energy – 2.68 ± 0.03 kJ, energy density – 18.4 J/mL, maximum temperature – 57.6 ± 0.03°C. Emulsions were left in the thermostatic cabinet at 20°C for 24 hours, to stabilize them. The computed composition of creams is presented in Table 1.

### 2.5 Analyses of creams

**2.5.1 Measurement of pH value and total acidity of creams**

The pH value of fresh emulsions (50 mL) was measured using a pH-meter FP20 (Mettler-Toledo Sp. z o.o., Warsaw, Poland) equipped with a glass calomel composite electrode. The standard buffer pH 4 and pH 7 solutions were used for electrode calibration. The total acidity of emulsions was determined by titration according to PN-78/A-86028. The arithmetic mean of the triplicate measurements was calculated.

**2.5.2 Determination of particles size distribution and dispersity index of creams**

The fat globules images were captured using an optical microscope (Delta Optical 100 TP, Minsk Mazowiecki, Poland). A droplet of cream was placed on the slide glass and covered with a coverslip and observed in a bright field. The dispersed particles of creams were observed at 600× magnification. The particles sizes were measured in ten pictures using a DLT-Cam Viewer software. The particle size distribution plots were prepared using MS Excel, and showing the relative frequency distribution and cumulative frequency distribution. The backgrounds of diagrams were presented in Table 1.

### Table 1 Composition of creams.

| Type of cream | Fat phase | Aqueous phase | Total fat (wt%) | Total protein (wt%) | Source of protein | Additional stabilizer |
|---------------|-----------|---------------|----------------|---------------------|------------------|----------------------|
| C             |           |               | 30.0           | 2.2                 | milk             | carrageenan          |
| MFS           | 30        | 70            | 31.5           | 2.6                 | soybean          | –                    |
| POS           |           |               | 30             | 70                  |                  |                      |
| OGS           |           |               | 31.5           | 2.6                 |                  |                      |

Explanations: C – sweet dairy cream (comparative sample), MFS – cream with milk fat, POS – cream with palm oil, OGS – cream with oleogel.

2.5.3 Particle size determination

The average particle size of the dispersed phase of creams was determined with a Turbiscan Lab apparatus (Formulation, France, Toulouse). The SMLS (Static Multiple Light Scattering) method is based on the measurement of the light intensity transmitted and backscattered by the sample, using a pulsating near-infrared light source with a wavelength of 880 nm. The average particle size was determined based on the BS value (at a height of 10–30 mm) and optical parameters of the emulsion, such as the volume fraction of the dispersed fraction, transmittance of the continuous phase, refractive indices of the continuous phase and the dispersed phase^{21,32}. The samples were scanned in three times for each of the duplicates (21 ± 2°C). The calculations were made with the TurbiSoft 2.3 software.

2.5.4 Viscosity measurement of creams

A viscosity test was carried out at increasing (every 10 r/min) shear velocity (from 10 to 250 r/min) using a Brookfield DV3T rotational viscometer (Brookfield Engineering Laboratories, Inc., Middleboro, USA). The samples (25 g) were tested under controlled conditions, using a cylindrical adapter and a DIN-85 spindle (equipment of the viscometer). The temperature was maintained at 20°C in all the experiments using a water bath. Viscosity curves were described by Ostwald’s mathematical model (τ = kγ^n), where: τ – shear stress (Pa), k – consistency index (mPas), γ – shear rate (s⁻¹), n – dimensionless flow index. Coefficients of variation of viscosity during shearing were computed^{33}. Measurements were made three times for each sample. The final result was the arithmetic mean of the obtained values.

2.5.5 Microrheological properties of creams

The microrheological characteristics of creams were analyzed according to our previous research^{27}. The Rheolaser Master (Formulaction, L’Union, France) was used and it operates using the dynamic multi-speckle diffusing-wave...
spectroscopy technique (MS-DWS). The light source was a coherent laser beam with a wavelength of 650 nm (NIR), and the detector – a CCD camera with a frequency of 27 Hz. The measurement results were recorded and compiled using Rheotest software. The microrheological parameters were computed based on MSD (Mean Square Displacement, nm²) curves in the function of decorrelation time (tdec). The Elasticity Index – EI (nm⁻²) is an inverse of the MSD value at the plateau in the mean tdec and corresponds to the elastic modulus (Pa). The MVI (nm⁻²) parameter is an inverse of the MSD at the slope in the high tdec and corresponds to the apparent viscosity at zero shear (mPas). The creams (20 mL flat bottom vials) were analyzed 24 h from their production. The “quick characterization” test was performed for 10 min at 20 ± 1°C (in triplicate for each sample).

2.5.6 LUMiSizer test

The physical stability of creams was also determined based on our previous studies[57, 59]. The LUMiSizer 612 dispersion analyzer (LUM GmbH, Germany) was used. It works based on the STEP (Space- and Time-resolved Extinction Profiles) technology that enables immediate detection of changes in light transmission across the entire length of the sample. The method is referred to as accelerated centrifugal stability analysis – CSA. The light source is multi-wavelength (near-infrared or blue). Detection is carried out by a CCD-line camera with above 2000 detectors. The measurement included the following settings: sample volume (0.5 mL), centrifugal force (4000 r/min), wavelength (870 nm), light factor (1.0), temperature (20 ± 1°C), transmission profiles recording time (10 s), the total duration of analysis (50 min). The results were elaborated using SEPView software. The increase of transmission level on the left side (top of the sample) or on the right side (bottom of the sample) of diagram means a decrease of particles concentration, which are sedimenting or floating, respectively. Based on the light transmission profiles as a function of time and position over the entire sample (cream) length, the phenomena of destabilization were defined, as well as the instability indexes and particles rates in the centrifugation field (at a height of 110–130 nm) were calculated.

2.6 Statistical analyses

As part of the statistical analysis of the test results, a one-way ANOVA was performed using: Shapiro-Wilk’s test for the normality of data, Levene’s test and Brown-Forsythe’s test for the equality of variances, sigma restricted parametrization for the equality of means, Tukey’s test (HSD) for the homogeneity of groups, at the significance level α = 0.05. Moreover, the strength of the correlation between the selected variables was calculated using the Pearson test[33]. The following correlation assessment criterion was adopted: excellent (ρ = ± 1.0), strong (± 0.8 ≤ ρ < ± 1.0), moderate (± 0.5 ≤ ρ < ± 0.8), weak (± 0.1 ≤ ρ < ± 0.5) and very weak (0.01 ≤ ρ < ± 0.1) [33]. The Statistica 13.1 software (TIBCO Software Inc., Palo Alto, CA, USA) was used.

3 Results and Discussion

3.1 Fat systems characteristics

The fatty acids composition of the tested lipids is shown in Table 2. The studied vegetable oils contained trace amounts of trans unsaturated fatty acids (TFAs). Trans isomers accounted for approx. 1.7% of the total fatty acids pool in milk fat (MF). In addition, MF had the highest content of SFAs (~67.3%), with approx. 13.2% of short-chain SFAs (C4-C12). Palm oil (PO) and the mixture of rapeseed oild with linseed oil (RO-LO) contained approx. 49.5% and 8.8% of SFAs, respectively. Oleogel did not differ significantly from the base oil in terms of fatty acids composition. It consisted of approx. 40% monounsaturated fatty acids (MUFAs) and 50% polyunsaturated fatty acids (PUFAs). The latter group was dominated by the α-linolenic acid from omega-3 group (~31%). Montoya et al. for palm oil[31], Kumar et al. for milk fat (ghee)[30], Kostik et al. for rapeseed and linseed oils[27] obtained similar results. In turn, Pehivanoglu et al. showed that the type of oil used determines the fatty acids composition of oleogels[30].

According to Codex Alimentarius (2001) the acid value for refined fats and oils should not exceed 0.6 mg KOH/g fat and the peroxide value – 10 meq. O₂/kg fat[30]. Palm oil (PO) and the mixture of rapeseed oil and linseed oil (RO-LO) met the mentioned above requirements. Oleogel (OG) obtained by structuring RO-LO with candelilla wax (CW) presented above 3-fold greater acid value, in comparison to the base oil mixture (Fig. 1A). It was found that the AV of oleogel was above the limit, if the oleogel was considered as a refined vegetable fat[30]. It was not caused by hydrolysis (absence of water), however by incorporation of a structuring agent – candelilla wax, which acid value equals approx. 18 mg KOH/g wax. Any increase in CW concentration will lead to a higher AV of oleogel. Moghtadaei et al. came to similar conclusions[40]. They demonstrated that an acid value of sesame oleogels significantly increased with the larger concentration of BW beeswax. According to Codex Alimentarius (2006), the maximum content of free fatty acids in milk fat ought to not exceed 0.4 wt% (as oleic acid), i.e. approx. 0.8 mg KOH/g fat, and the PV ~ 0.6 meq. O₂/kg fat[41]. The fresh milk fat was characterized by a level of acid value in accordance with guidelines, and it was noted that this value was not statistically significantly higher as compared to the OG. After 30 days of fats storage at 20°C, there was a relevant increase in the degree of hydrolysis (ρ ≤ 0.05) merely in the case of milk fat (approx. 2.5 times in relation to the “0” day), exceeding the permissible
The presence of short-chain fatty acids, which are more susceptible to hydrolysis, and water as a residue from industrial processing were crucial. All lipids, except MF (PV > 0.6 meq. O₂/kg fat), fulfilled the requirements in the range of permissible peroxide values. According to Musiy & Tsisyryk, increasing the degree of hydrolysis can accelerate the oxidation of fats. In addition, free unsaturated fatty acids oxidize first. The RO-LO and OG samples had more than two times larger value of this parameter (p ≤ 0.05) compared to the PO (0.63 meq. O₂/kg oil). The high content of linseed oil, as a rich source of polyunsaturated fatty acids, explains the greater degree of oxidation in RO-LO and the tendency to form hydroperoxides and peroxides. However, the important information, from a technological point of view, is the lack of difference (p > 0.05) between the PV of oleogel and its base mixture of oils. The oleogelling process (80°C for 10 min, sonication for 1 min) did not contribute to the in-

Table 2: Fatty acids composition of fat systems.

| Fatty acid (carbon atoms : double bonds) | Type of fat system |
|-----------------------------------------|-------------------|
|                                         | MF    | PO    | RO-LO | OG    |
| C 4:0                                   | 3.95 ± 0.16   | ND    | ND    | ND    |
| C 6:0                                   | 2.27 ± 0.13   | ND    | ND    | ND    |
| C 8:0                                   | 1.08 ± 0.04   | ND    | ND    | ND    |
| C 10:0                                  | 2.56 ± 0.04   | ND    | ND    | ND    |
| C 12:0                                  | 3.37 ± 0.10   | ND    | ND    | ND    |
| C 14:0                                  | 10.35 ± 0.52  | 0.92 ± 0.05 | ND    | ND    |
| C 14:1 (cis-9)                          | 2.23 ± 0.03   | ND    | ND    | ND    |
| C 16:0                                  | 33.08 ± 0.04  | 43.62 ± 0.42 | 5.17 ± 0.14 | 5.02 ± 0.07 |
| C 16:1 (trans-9)                        | 0.27 ± 0.02   | ND    | ND    | ND    |
| C 16:1 (cis-9)                          | 1.71 ± 0.05   | ND    | 0.13 ± 0.01 | 0.14 ± 0.01 |
| C 17:0                                  | 0.60 ± 0.06   | ND    | 0.08 ± 0.01 | 0.07 ± 0.01 |
| C 17:1 (cis-10)                         | 0.13 ± 0.01   | ND    | 0.06 ± 0.01 | 0.10 ± 0.01 |
| C 18:0                                  | 10.09 ± 0.16  | 4.50 ± 0.08 | 2.81 ± 0.03 | 2.71 ± 0.04 |
| C 18:1 (trans-9)                        | 1.32 ± 0.07   | ND    | ND    | ND    |
| C 18:1 (cis-9)                          | 23.32 ± 1.08  | 40.4 ± 0.41 | 39.53 ± 0.57 | 38.61 ± 0.97 |
| C 18:2 (trans-9,12)                     | 0.11 ± 0.01   | ND    | 0.06 ± 0.01 | 0.10 ± 0.01 |
| C 18:2 (cis-9,12)                       | 1.82 ± 0.04   | 9.61 ± 0.23 | 17.59 ± 0.18 | 17.08 ± 0.39 |
| C 18:3 (cis-6,9,12; cis-9,12,15)        | 1.21 ± 0.04   | 0.26 ± 0.01 | 32.42 ± 0.42 | 31.23 ± 0.24 |
| C 20:0                                  | ND            | 0.36 ± 0.01 | 0.37 ± 0.02 | 0.35 ± 0.01 |
| C 20:1 (cis-11)                         | ND            | 0.10 ± 0.01 | 0.74 ± 0.02 | 0.75 ± 0.01 |
| C 20:2 (cis-11,14)                      | ND            | ND    | ND    | 0.58 ± 0.02 |
| C 22:0                                  | ND            | 0.09 ± 0.01 | 0.24 ± 0.01 | 0.22 ± 0.01 |
| C 22:1 (cis-13)                         | ND            | ND    | 0.13 ± 0.01 | 0.15 ± 0.01 |
| C 22:2 (cis-13,16)                      | ND            | ND    | ND    | 0.96 ± 0.04 |
| C 24:0                                  | ND            | ND    | 0.12 ± 0.01 | 0.13 ± 0.01 |
| C 24:1 (cis-15)                         | ND            | ND    | ND    | 0.15 ± 0.01 |
| ∑ SFAs                                  | 67.34 ± 0.83c | 49.47 ± 0.52b | 8.78 ± 0.34b | 8.46 ± 0.72c |
| ∑ MUFAs                                 | 27.39 ± 1.05a | 40.5 ± 0.45b | 40.58 ± 0.96b | 39.89 ± 0.79b |
| ∑ PUFAs                                 | 3.03 ± 0.37c  | 9.87 ± 0.29b | 50.01 ± 1.02b | 49.85 ± 0.94c |
| ∑ TRANS                                 | 1.70 ± 0.13b  | ND    | 0.06 ± 0.01c | 0.10 ± 0.01c |

ND – non detectable, i.e., < 0.05 %
a, b, c... - designations of homogeneous groups in verses, p ≤ 0.05
Explanations as below Table 1.

limitations (Fig. 1A). The presence of short-chain fatty acids, which are more susceptible to hydrolysis, and water as a residue from industrial processing were crucial.

All lipids, except MF (PV > 0.6 meq. O₂/kg fat), fulfilled the requirements in the range of permissible peroxide values. According to Musiy & Tsisyryk, increasing the degree of hydrolysis can accelerate the oxidation of fats. In addition, free unsaturated fatty acids oxidize first. The RO-LO and OG samples had more than two times larger value of this parameter (p ≤ 0.05) compared to the PO (0.63 meq. O₂/kg oil) (Fig. 1B). The high content of linseed oil, as a rich source of polyunsaturated fatty acids, explains the greater degree of oxidation in RO-LO and the tendency to form hydroperoxides and peroxides. However, the important information, from a technological point of view, is the lack of difference (p > 0.05) between the PV of oleogel and its base mixture of oils. The oleogelling process (80°C for 10 min, sonication for 1 min) did not contribute to the in-

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increased primary oxidation products content.

Moreover, a significant increase in the PV was observed for each of the fat or oil samples during the 30 days of storage at 20°C. The greatest deterioration of quality was noticed for RO-LO (approx. 3 times higher PV), while the smallest changes occurred in palm oil (approx. 1.8 times higher PV) (Fig. 1B). The creation of a three-dimensional structure in the oleogelation process with the use of a structuring agent (candelilla wax) contributed to the slight deceleration of oxidative changes. However, it was not sufficiently enough to maintain level of PV comparable to palm oil. The result was corroborated by Lim et al., who studied the PV of rapeseed oil, wax-structured rapeseed oleogels, and peroxide values – PV (B) of fats/oils at 0 day or 30 days of storage. Explanations: MF – milk fat; PO – palm oil; OG – oleogel; RO-LO - rapeseed oil and linseed oil mixture at 1:1 ratio and peroxide values – PV (B). The peroxide value enlargement was the greatest in the case of oleogels with candelilla wax and was over three times lower than the PV of oleogels after 18 days. In return, Öğütçu & Yılmaz showed no significant changes in the number of primary oxidation products in oleogels based on hazelnut oil with sunflower wax or carnauba wax in the amount of 3, 7, 10 wt%, stored at 20°C for 30 days. According to the authors, this was mainly carried out by the high oleic acid content in hazelnut oil. In the studies of Barroso et al., it was demonstrated that the formation of a three-dimensional structure from cold-pressed linseed oil with a 10 wt% different gelling agent (berry wax, sunflower wax, and monoacylglycerols) was not sufficient to protect unsaturated bonds from singlet oxygen and reduce the primary degree of oxidation of the oil during 30 days of oleogels storage at 25°C.

As shown in Table 3, the investigated fats differed significantly in terms of the determined physical parameters. The lowest slip melting point (SMP) was noted for milk fat (approx. 30°C), and the highest for oleogel (OG). Palm oil had a higher slip melting point (by approx. 4°C) than MF, despite the lower content of saturated fatty acids. Thus, no significant correlation was found in the Pearson test between the physical parameters of the tested fat systems (p > 0.05). Devi and Khathar received similar SMP values for palm oil. Furthermore, they indicated a correlation between the fatty acids composition and the slip melting point (SMP) of fats. The SMP of fat depends on both the degree of unsaturation, and the length of the carbon chains of fatty acids – the shorter carbon chain means the lower melting point, and this can explain the lower SMP for MF than for PO. Palmitic acid (C16) dominates in palm oil, while milk fat contains a lot of short- (C4-C12) and medium-chain (C12-C14) SFAs. In addition, the SMP value is determined by the quantity of gelling substance, wherein increasing its concentration allows for a higher slip melting point of oleogel, thereby improving their oil binding ability. The tested oleogel was likewise characterized by the greatest capacity for retaining the liquid fraction in the structure during centrifugation (Table 3). In view of the
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above, it can be concluded that the structuring of liquid vegetable oil with a small amount of a gelling agent is a more effective method of obtaining a stable fat system than increasing its SFAs content.

3.2 Physicochemical properties of creams

The fat system type did not have a significant influence ($p > 0.05$) on the acidity of the emulsions based on the soy drink. Sweet dairy cream (comparative sample) showed a remarkably lower pH value (by approx. 3%) and higher acidity (by approx. 19%) compared to the experimental samples. These formulations did not differ in terms of acidity ($p > 0.05$) from the soy drink (Table 4). The gained results were within the range of parameters provided for fresh cow’s milk or the so-called soy drink. According to Ismail Abou-Dobara et al., the total acidity of this type of product (fresh) not exceeding 8 °SH, and the pH not lower than 6.6 proves their good quality. Thus, the sonication process, applied for emulsions production, caused no deterioration in the quality of the aqueous phase.

The most stable O/W emulsions are monodispersed, single-fraction systems, which means containing the uniform (preferably small) sizes of dispersed particles in throughout the whole volume of the sample. All creams were characterized by unimodal distribution of lipid dispersed particles (one local extreme of the curve). The relative frequency distribution curve of particles size in POS is slightly lower and wider at the base, compared with the other emulsions (Fig. 2). Moreover, this cream exhibited the highest value of dispersity index – approx. 1.16 (Fig. 3B). Therefore, the POS can be the least physically stable formulation.

### Table 4 Values of pH and total acidity of creams.

| Type of sample | pH (-) | Total acidity (° SH) |
|----------------|--------|----------------------|
| S              | 6.94 ± 0.04$^a$ | 5.62 ± 0.06$^{ab}$ |
| C              | 6.68 ± 0.03$^a$ | 6.66 ± 0.04$^a$ |
| MFS            | 6.91 ± 0.02$^b$ | 5.73 ± 0.03$^b$ |
| POS            | 6.91 ± 0.02$^{ab}$ | 5.61 ± 0.05$^b$ |
| OGS            | 6.91 ± 0.02$^b$ | 5.67 ± 0.05$^b$ |

a, b, c... - designations of homogeneous groups in columns, $p \leq 0.05$

Explanations as below Table 1; S – soy drink

### Fig. 2

Particles size distribution in dairy cream (A), and creams with milk fat (B), palm oil (C) or oleogel (D)

a, b, c... - designations of homogeneous groups, $p \leq 0.05$
Emulsions containing MF or OG were not dissimilar \( p > 0.05 \) in the mean particle size of the internal phase, compared to the C \( (1.74 \mu m) \). It was found that the emulsification of the soy drink with the aforementioned lipids by ultrasonic homogenization for 1 min \( (80\% \text{ amplitude}, 100\% \text{ pulse}) \) made it possible to obtain similar sizes of dispersed particles to the sizes of the milk fat micelle in sweet cream stabilized with carrageenan. Merely the POS belonged to a different homogeneous group, in comparison with dairy cream \( \text{Fig. 3A} \). The presence of a small amount of soybean oil \( \text{added with the aqueous phase} \) in the vegan creams can have exaggerated the results for these formulations \( \text{Table 1} \). Kaltsa \textit{et al.} analyzed the effect of ultrasonic treatment on the quality of O/W emulsion with the use of virgin olive oil, stabilized with whey protein isolate, with the addition of xanthan gum. Emulsions homogenized for 1 min, at an amplitude of 80\%, were characterized by the size of the oil droplets at the level of 1.55–1.75 \( \mu m \). Kowalska \textit{et al.} obtained the O/W emulsions \( (30:70) \), stabilized with sunflower lecithin and prepared with a mechanical homogenizer, with mean sizes of dispersed particles in the range of 3.2–5.0 \( \mu m \). The Authors reported the impact of lipid type used \( \text{a chemically modified mixture of calf tallow and pumpkin seed oil (CT:PO) in various proportions} \), and type of a thickener \( \text{maltodextrin, xanthan gum, and/or microcrystalline cellulose} \) on the average size of dispersed particles. The lowest values of this parameter showed the samples containing CT:PO 1:3 and CMC 32.

### 3.3 Rheological properties of creams

The fat phase-type affected the microrheological behavior of the examined emulsions. The lowest macroscopic viscosity was perceived for the POS sample and the dairy cream \( \text{C} \). The MFS and OGS had an approx. 43 and 32\% higher values of the macroscopic viscosity index \( \text{MVI} \), respectively, compared to the dairy cream – 1.66 \( \times 10^{-2} \text{ \text{nm}^{-2} \text{m}^{-1}} \).
Fig. 5  Creams’ viscosity curves obtained at increasing shear rate.

Explanations as below Table 1

Fig. 4A. The same dependence occurred for the mean values of the elasticity index (EI), although the differences were smaller and amounted to approx. 23% (Fig. 4B). The mean values of the microrheological parameters MVI and EI were strongly positively correlated

\( \rho = 0.98, R^2 = 0.96, t = 6.99, p = 0.02 \).

It can be assumed that the higher viscosity and elasticity of the OGS could result from the highest slip melting point of oleogel, and in the case of the MFS – from the highest content of saturated fatty acids. Scott et al. researched the reformulation of sweet cream, using different fractions of anhydrous milk fat in the amount of 20% w/w and subjecting them to emulsification with skim milk. The authors found that the emulsions containing the medium melting fraction (29.3% palmitic acid and 9.6% stearic acid) showed greater apparent viscosity and storage stability than natural cream.

Figure 5 shows the viscosity curves of emulsions. The viscosity of samples decreased with the shear rate increased, hence, the strong negative correlation between these quantities for each emulsion separately, i.e.: C – \( \rho = -0.85, R^2 = 0.72, t = -7.78, p = 0.00 \); MFS – \( \rho = -0.88, R^2 = 0.77, t = -8.78, p = 0.00 \); POS – \( \rho = -0.93, R^2 = 0.86, t = -11.98, p = 0.00 \); OGS – \( \rho = -0.90, R^2 = 0.81, t = -9.88, p = 0.00 \). All emulsions, regardless of fat phase-type, were identified as the non-Newtonian shear-thinned (pseudoplastic) fluids. This is consistent with the results of Woźniak et al., who studied the emulsions (30/70 w/w), stabilized with xanthan gum and scleroglucan, and containing an enzymatically modified blend of sheep tallow and hemp seed oil. Based on the analysis of the viscosity curves and the determined rheological parameters, it was found that the emulsion with oleogel (OGS) showed the greatest similarity to the MFS. The strong positive correlation between MVI (Fig. 3A) and k (Table 4) occurred \( \rho = 0.97, R^2 = 0.95, t = 5.94, p = 0.03 \). Interestingly, with the greater viscosity coefficient of the sample, its degree of shear-thinning was slightly increased (Fig. 5, Table 5). As shown in Table 5, it was confirmed by the values of viscosity coefficients of variation during emulsion shearing. Similar findings can be seen in the study by Quintana-Martinez et al., who analyzed rheological properties of the O/W emulsions with different concentrations of guar gum (as a thickener) and lecithin (as an emulsifier). The greater addition of thickener led to an increase of apparent viscosity of emulsions, simultaneously to a higher degree of their shear-thinning.

In addition to the above, there was a strong negative correlation between mean values of viscosity coefficients and flow indicates of emulsions \( \rho = -0.99, R^2 = 0.98, t = -10.67, p = 0.01 \).

3.4 Physical stability of creams

The light transmission profiles for all formulations are shown in Fig. 6. The evolution of these profiles in centrifugation time for all emulsions proves the initiation of the creaming phenomenon, i.e. flotation of lipid phase particles – reduction of particles concentration at the bottom of the system. The control sample (dairy cream) was characterized by different shapes of this profile. The increase of light transmission was lower. However, it included a larger volume of the measured sample. The kinetics of emulsion stability changes as a function of time (Fig. 7) had an exponential course for the emulsion based on the soy drink. The

Table 5  Rheological parameters and viscosity coefficients of variation during shearing of creams.

| Type of sample | Vc (%)  | k (mPa s)     | n (-)       | R²  |
|---------------|---------|---------------|-------------|-----|
| C             | 17.9 ± 0.2a | 68.8 ± 0.9b  | 0.817 ± 0.007b  | 0.98 |
| MFS           | 20.8 ± 0.3c | 93.0 ± 0.7c  | 0.771 ± 0.009g | 0.98 |
| POS           | 15.2 ± 0.2c | 65.0 ± 1.0c  | 0.835 ± 0.011b | 0.97 |
| OGS           | 20.5 ± 0.3c | 91.1 ± 0.3c  | 0.776 ± 0.008a | 0.98 |

a, b, c... - designations of homogeneous groups in columns, \( p \leq 0.05 \)

Explanations as below Table 1; Vc – viscosity coefficient of variation; k – consistency coefficient (mPa s), n – flow index (-), R² – coefficient of determination

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increase in instability index value for the comparative sample was linear up to 2400 s of the analysis duration. The alterations in the stability, which differentiated the samples, occurred after 1200 s of centrifugation. Moreover, there were strong positive correlation between centrifugation time and total instability index of each emulsion separately, as following: C – $\rho = 0.96$, $R^2 = 0.92$, $t = 58.37$, $p = 0.00$; MFS – $\rho = 0.97$, $R^2 = 0.93$, $t = 65.04$, $p = 0.00$; POS – $\rho = 0.96$, $R^2 = 0.92$, $t = 59.73$, $p = 0.00$; OGS – $\rho = 0.97$, $R^2 = 0.93$, $t = 63.54$, $p = 0.00$. To sum up, the results proved that the time of exposure to the centrifugal force had a significant impact on the stability of the emulsions.

The final effects of the emulsion stability changes illustrated the values of the total instability index and the rate of the dispersed phase particles in the centrifugation field, shown in Table 6. If the instability index was lower, the emulsion was more stable. Generally, the decrease of the total instability index value increased the velocity of the

| Type of sample | Total instability index (-) | Particles rates (µm/s) |
|---------------|-----------------------------|------------------------|
| C             | 0.023 ± 0.002$^a$           | 0.568 ± 0.022$^a$      |
| MFS           | 0.033 ± 0.002$^b$           | 0.547 ± 0.016$^a$      |
| POS           | 0.052 ± 0.003$^c$           | 1.194 ± 0.019$^b$      |
| OGS           | 0.031 ± 0.003$^b$           | 0.554 ± 0.012$^a$      |

Table 6. Total instability indexes and particles rates of creams.

a, b, c... - designations of homogeneous groups in columns, $p \leq 0.05$

Explanations as below Table 1.

Fig. 7 Kinetics of instability indexes changes in function of centrifugation time of creams.
Explanations as below Table 1.
particles. However, there was no significant correlation in accordance with the Pearson test \( (p > 0.05) \). The POS exhibited the lowest stability (Fig. 7, Table 6), which could be a consequence of inferior physical stability of palm oil (Table 3), which enhancement the risk of liquid fraction migration in the dispersion system likewise. The higher macroscopic viscosity of MFS and OGS (Fig. 4A, Fig. 5) has chiefly contributed to the limitation of the creaming phenomenon. It can be assumed that the high physical stability of the comparative sample was related to the presence of carrageenan as a stabilizing substance in its composition (Table 1). In other studies, low-fat O/W emulsions containing lipids structured with ethyl cellulose, stabilized with soy lecithin (5.0\% w/w) and guar gum (0.6\% w/w), were characterized by comparatively high stability to the control sample (30 g milk fat as fat phase per 100 g emulsion) – the value of the total instability index was 0.001-0.004 after 24 h (20°C) from their production. In emulsions with oleogels, the flotation (creaming) mechanism of fat phase droplets mainly occurred, while in the control sample, the phenomenon of sedimentation of particles with a higher specific weight than fat progressed. Taking into account the use of ethyl cellulose, in which case the high-temperature treatment is necessary (at least 140°C), and another oil structuring agents, underlining such aggressive conditions of processing (approx. 80°C), the application of such wax in production of food emulsions is more justified, both from a technological and a nutritional point of view.

4 Conclusion

The type of applied lipid affected the quality of the creams based on the soy drink. Structuring the vegetable oil with a small amount of gelling agent (3\% w/w) resulted in obtaining a fat system with greater physical stability than fat with a high content of saturated fatty acids (milk fat). Simultaneously, the oleogelation process (with the candelilla wax usage) limited the occurring oxidative changes of the oil enclosed in the formed structure. It has been indicated that it is possible to get a vegan emulsion with physical properties similar to commercial UHT dairy cream. Finally, the emulsion with oleogel was characterized by greater durability (resistance to the centrifugal force) than the emulsion with palm oil, which gives a major application possibility of these structured lipids as substitutes for conventional solid fats. It has also been proved, that it is achievable to obtain emulsions with greater stability without the addition of thickeners, in consequence of the presence of soy protein (emulsifier) in the aqueous phase (soy drink).

Contributions

Designed research, performed research, analyzed data, wrote the manuscript – J.S.; reviewed and edited, final approval – A.Ż., M.K.; funding acquisition – M.K., K.G. All authors have read and agreed to the published version of the manuscript.

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Conflicts of Interest

The authors do not declare conflict of interest.

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