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APPLICATION OF TURBISCAN LAB TO STUDY THE EFFECT OF EMULSIFIER CONTENT ON THE STABILITY OF PLANT ORIGIN DISPERSION

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The effect of emulsifier volume on emulsion system stability of plant origin being the basis of diet supplements for animals in winter season was analyzed. For this purpose, measurements of the backscattered light intensity as the function of the measuring cell height were conducted with a Turbiscan LAB optical analyzer. System stability was analyzed on the basis of Turbiscan Stability Index values. A Helos laser analyzer and a Nikon Eclipse E400 POL optical microscope were used to investigate drop size distribution and analyze microscopic pictures. It was shown that emulsion with 10% (w/w) of the emulsifier was the most stable one.

Keywords: emulsion, polysorbate 80, backscattered light, droplet size, stability

1. INTRODUCTION

Formation of emulsion systems and maintaining that state for the time required for the course of desirable processes is the basic operation in almost each (bio)technological process (Vermeir et al., 2016). Attainment of such a state is not simple and is possible only when surface tension forces between phases are negligible. Otherwise, drops of the dispersed phase merge in a very short time. As a result, the emulsion in its structure may comprise two or more continuous phases (coalescence) (Bernewitz et al., 2011). To prevent that, third substances called emulsifiers (EM) should be used. Owing to their structure, they are located on the border of phases taking over their roles, causing a drop of surface tension or its decay at the same time (Chen et al., 2017). Moreover, according to the Stokes's law (Chanamai and McClements, 2000; Wassenius et al., 2001), particle migration rate for a monodisperse diluted system of spherical particles is directly proportional to the square of particle (drop) diameter of the dispersed phase, the difference in density of both phases, and acceleration having an effect on the emulsion, while inversely proportional to the viscosity of the continuous phase. Thus, from the engineering point of view, lowering the dispersed phase migration speed can be the result of many process and operating parameters, the incorrect selection of which may result in occurrence of undesired phenomena – creaming (Abismaıet al., 2000; Celia et al., 2009; Kowalska et al., 2015), sedimentation (Akther et al., 2008), or phases inversion (Basaran and Tasdemir, 2014; Li et al., 2016; Zawala et al., 2017) – leading finally to system destabilization. Hence, the engineering aspect is closely related with determination of mixing power (suitable frequency of revolutions) necessary to ensure the required system uniformity, which usually increases with the rise of the continuous phase kinematic

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viscosity (Rosdi et al., 2018). Therefore, emulsifier volume and revolution frequency are the crucial factors, which in the most meaningful way affect the stability of generated emulsions. Their optimum selection is strictly connected with obtaining the most advantageous system characteristics taking into account drop size distribution. The bigger shredding of particles in dispersion system, and the more limited particle size distribution (slender normal distribution curve), the more stable system can be expected. Consequently, it may be reflected in the physiochemical properties of obtained dispersion systems (viscosity, density, consistence, fluidity, color), or – for foodstuffs – changes in their taste and flavor. The last aspect connected with food processing is particularly important, as it may facilitate assimilation of nutrients providing with nutritive value necessary for body functioning. It should be strongly emphasized that most of emulsions functioning in nutrition of people and animals belong to unstable systems showing a tendency to spontaneous distribution of phases and loss of their desired organoleptic properties.

Hence, in the present paper the effect of emulsifier content on the stability of carefully selected composition of herb oils (mint and eucalyptus) as well as vitamin A in the conditions of variable revolution frequency, at different homogenization times, was studied using a Turbiscan-LAB (Formulaction, France) stability analyzer based on multiple light scattering technology. The composition under consideration was produced by a Polish company from Kuyavian–Pomeranian province and it provides supplementation of animal diet to support their immune system, increase breeding and economic effects.

The addition of mint oil is justified due to its disinfecting, relaxant and soothing properties. It also cleans the respiratory tract of any residual secretion. On the other hand, eucalyptus oil refreshes and shows stimulating, antiviral, anti-inflammatory and analgesic effects (Köteles et al., 2018). The role of vitamin A is closely connected with the vision process (Abe-Matsumoto et al., 2018). Polysorbate 80 was used as the emulsifier. It is usually applied in the process of ice-cream making as well as to prevent deproteinization of fat drops in milk (Rudnicka et al., 2017).

Analysis showed that apart from nutrients providing valuable diet supplements for an organism, it is also crucial to produce the right texture of the final product to ensure its easy digestibility. The increase of emulsifier content makes the system more homogeneous (Zheng et al., 2018). Such an approach is right. However, as shown in this paper, the stability of a system should not be assumed without experimental verification and analysis of drop size as the key factor that affects emulsion properties, especially emulsion stability (Ghosh and Rousseau, 2009).

2. EXPERIMENTAL STUDIES

2.1. Materials

In order to prepare water compositions, the following components were used: 1) distilled water, 2) nonionic emulsified called Polysorbate 80 (polyoxyethylene sorbitan monooleate), being the mixture of polyoxyethylene derivatives of sorbitan and oleic acid and occurring in the form of viscous, water-soluble, yellow liquid of the density (298 K) 1100 kg·m^{-3}, dynamic viscosity coefficient (298 K) 0.40 Pa·s (Pharma – Cosmetic Sp. z o.o./Fagron Sp. z o.o.), 3) vitamin A, density (293 K) – 920 kg·m^{-3} (Interforum Pharma Sp. z o.o., Ceacow), 4) natural ethereal eucalyptus oil, density (293 K) – 911 kg·m^{-3}, refractive index (293 K) – 1.461, optical rotation (293 K) – 1.1°, solubility 1:5, ignition point 326 K (Avicenna-Oil, Wrocław), 5) natural mint oil, density (293 K) – 901 kg·m^{-3}, flash-point > 573 K, refractive index – 1.467 (Interforum Pharma Sp. z o.o., Cracow).

2.2. Measurement methods

2.2.1. Sample preparation

The stability of three liquid emulsion systems at a temperature of 298K and the composition presented in Table 1 was tested. The systems were characterized by a fixed content of eucalyptus oil (OE), mint oil (OM), vitamin A (VitA) as well as a fixed volume of emulsifier (EM) and water (W).

Table 1. Percentage composition of emulsion systems

Sample No.	EM	OE	OM	VitA	W
1	5	18	0.9	0.2	75.9
2	10				70.9
3	15				65.9

The total volume of each prepared sample was 100 cm^3. The prepared samples were homogenized with a laboratory homogenizer H 500 (Pol-Eko-Aparatura, Wodzisław Śląski, Poland) for 20 min., at the temperature of 298 K and at three different revolution frequencies of 2×10^3, 8×10^3 and 15×10^3 rpm. The homogenizer was equipped with a knife composed of a rotor and a stator located at the height of $h/D = 1/3$ from the bottom of the tank, where h and D represent height and dimension of the tank, respectively.

2.2.2. The principle of Turbiscan LAB measurement

Turbiscan LAB was employed to quantitatively and objectively characterize the stability of dispersion system samples without destruction, avoid the interference of subjective factors and show the cause of instability (aggregation or migration). Turbiscan LAB has a detection head which moves up and down along a flat-bottomed glass cylindrical cell (Fig. 1). The detection head is composed of a pulsed near-infrared light source (wavelength Λ = 850 nm) and two synchronous detectors. The transmission detector receives light which goes through the sample (0° from the incident beam), while the backscattering detector receives light scattered by the sample at 135° from the incident beam. The angle of 135° was chosen so as to be

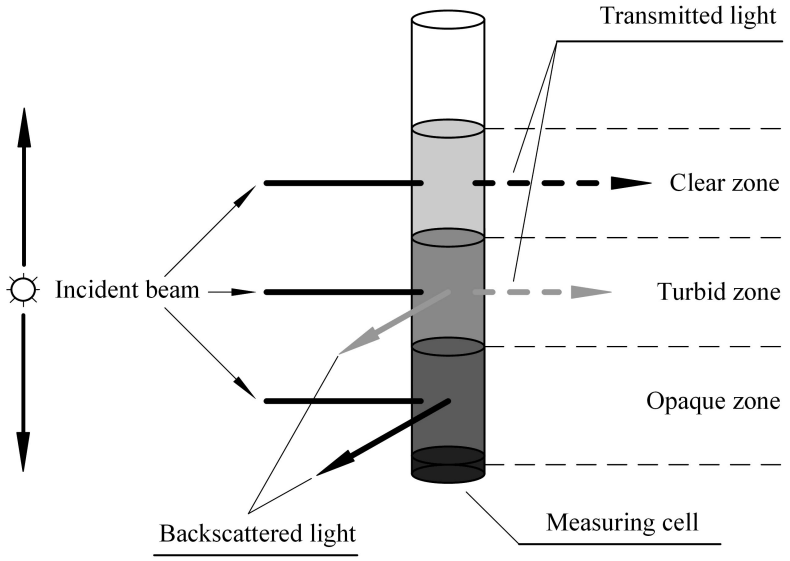

Fig. 1. Operation principle of Turbiscan LAB

outside the coherent backscattering cone (the cone angle θ_c of the coherent scattered intensity scales as Λ/λ^* (Wolf et al., 1988), where λ^* is the photon transport length). Turbiscan LAB device was described more in-depth by Bru et al. (2004), Mengual et al. (1999), and Snabre and Arhaliass (1998).

The analyzed emulsion was placed in a cylindrical glass cell. The optical reading head scanned the length of the sample (about 50 mm) acquiring backscattering data every 40 μm as a function of the distance along the axis of the tube and time (transmitted light was neglected because the emulsion is opaque).

Backscattered light (BS) and Turbiscan Stability Index (TSI) are used to quantify the stability of samples. The former summarizes all variations in each sample and gives a unique number that reflects the destabilization of a given sample. The calculation method of this coefficient is as follows (Wiśniewska, 2010; Zheng et al., 2018):

$$\mathrm{TSI} = \sqrt{\frac{\sum_{i=1}^{N}(x_i - x_m)^2}{N-1}} \tag{1}$$

where x_i $(i = 1, \ldots, N)$ is the mean backscattering, x_m is the mean value of x_i, and N is the number of scans.

The smaller the TSI value, the more stable the sample.

Samples were scanned for 6 days, for 5% (w/w) of the emulsifier content, and for 4 days in case of emulsions with emulsifier content of 10 and 15% (w/w).

2.2.3. Statistical analysis of data

Drop size distribution (DSD) in the emulsion was determined using a laser light diffraction analyzer HELOS (Sympatec GmbH, Clausthal-Zellerfeld, Germany). Data analysis was carried out with Matlab Statistic Toolbox (Mathworks Inc., Natick MA, USA) to determine statistical estimates and confirm the correctness of the performed prognosis. Analysis of DSD is pivotal in case of emulsion destabilization caused by aggregation (flocculation, coalescence) of particles that make up the dispersed phase.

2.2.4. Structure of emulsions – optical micrographs

Structure of emulsions was investigated using a polarization optical microscope Nikon Eclipse E400 POL (Precoptic Co., Poland (Camera Nikon type DS-Fi2, Emaging software NIS-Elements 4.0 for Windows)). The prepared emulsion was placed on a microscopic slide. A cover slip was placed on the sample. No air or bubbles were trapped between the sample and the cover slip.

3. ANALYSIS OF RESULTS AND DISCUSSION

Based on the characteristics of real emulsion state tests conducted with Turbiscan LAB, curves were obtained presenting the percentage share of BS, being the functions of the measuring cell height (h) and time. Experimental data made it possible to overlay next states of the emulsion, which enabled determination of stability or finding its loss. Overlaying of the curves obtained as a result of subsequent sample scanning justifies their high stability. On the other hand, the variable course of the curves points to the system instability.

Figure 2 presents changes in BS intensity as the function of the measuring cell length and the time of its scanning for the content of emulsifier amounting to 5% (above mentioned). The backscattering profile courses show that the addition of emulsifier in the quantity of 5% (w/w) does not stabilize the system in the expected manner resulting in differentiation of BS curves along the measuring cell irrespective of the scanning time. The presence of areas differing from each other with intensity of BS in the sample is clearly marked here.

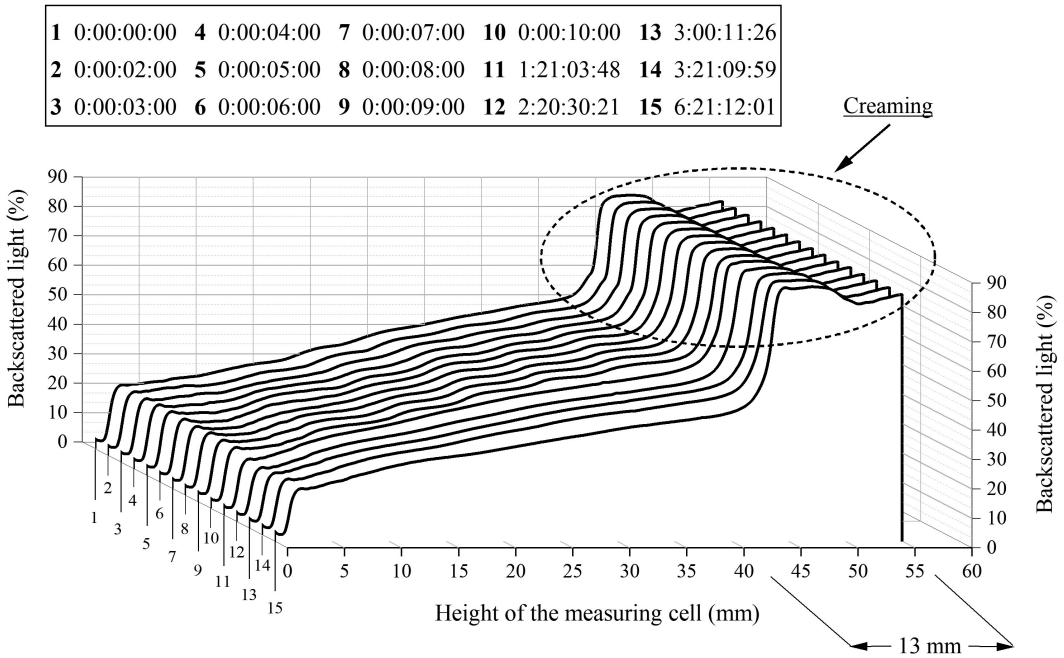

Fig. 2. Backscattered light intensity as a function of the measuring cell height and time for EM contents of 5% (w/w)

Up to the height of the measuring cell equal to 40 mm, a slight increase of BS intensity is shown (up to 50%), caused by migration of particles to the upper areas of the measuring cell (creaming). As a consequence, a sudden increase of BS intensity from 50% for h = 40 mm up to 80% for h = 43 mm is shown, which reveals the lack of system stability.

Stability may be ensured by increasing the volume of the added emulsifier, in particular of Polysorbate 80, which is shown in Fig. 3 presenting TSI values as the function of emulsifier content and scanning time. These dependencies have been drawn up based on the relation of BS vs. h illustrated in Fig. 2, for 5% (w/w) of EM and in Figs. 4 and 5 drawn up for EM added in the amount of 10% and 15% (w/w), respectively.

Figure 3 illustrates that the addition of emulsifier lowers TSI, which take values from 21.77 to 22.86, for emulsifier content of 5% (w/w), from 5.15 to 7.24, for the emulsifier content of 10% (w/w), and from 2.00 to 3.66 for the content amounting to 15% (w/w). Thus, while tracing TSI changes, it can be found that all the emulsions are stable. It can be justified by differences in TSI values lower than 0.4 (Manca et al., 2016), while the increase of the emulsifier content (decrease of TSI values) makes the emulsions more stable. However, TSI values give only the first look of sample stability and are not always sufficient to consider a system as stable.

Comparing the backscattering profiles presented in Figs. 4 and 5 for the mass contents of emulsifier amounting to 10% and 15% it can be found that in both cases the shorter the exposure time, the less stable the system (lines 1 and 2), showing higher BS intensity values by about 50%, and from 29% to 33%, respectively.

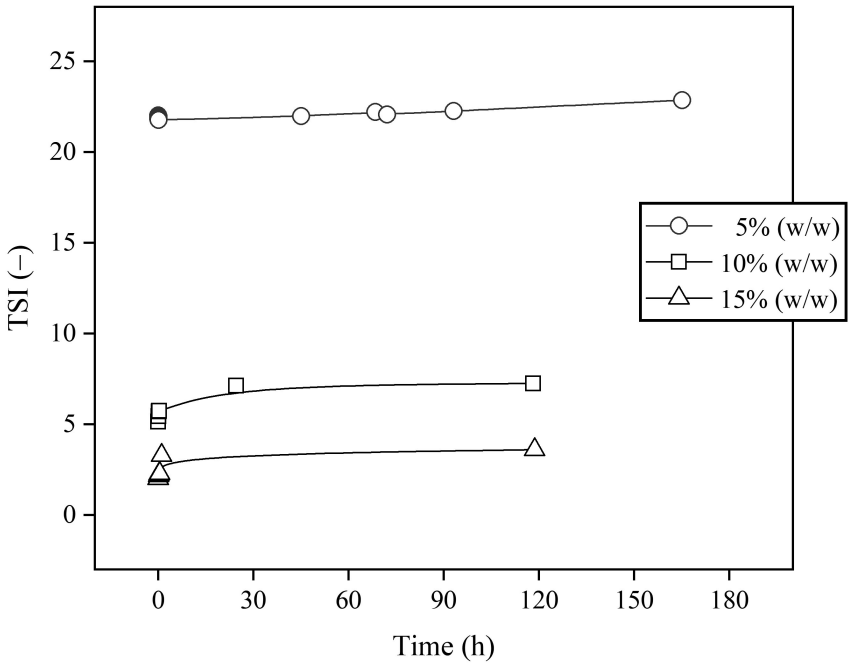

Fig. 3. Effect of EM content and storage time on TSI values

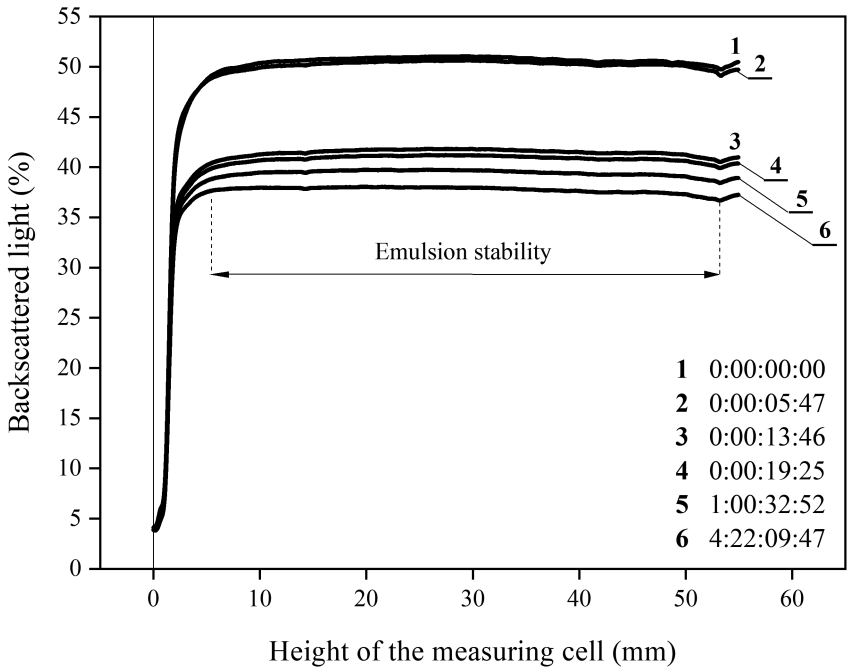

Fig. 4. Backscattered light intensity as a function of the height of a measuring cell and time for EM content of 10% (w/w)

As the time progresses – after 13 min and 18 min, respectively – the level of backscattering decreased noticeably over the total length of the sample for EM content of 10% (w/w) (Fig. 4), and in the middle of the vial for EM content of 15% (w/w) (Fig. 5), making the emulsion more stable. This phenomenon will affect the average diameter of particles in the sample resulting in a decrease of BS intensity from 37% to 40% for the EM content of 10% (w/w), and from 19% to 22% (Figs. 4 and 5 lines 3–6) for the EM content of 15% (w/w). As shown below, the lower level of BS intensity may be the consequence of the growth of dispersed phase drop size. This diameter will increase as coalescence or flocculation are global phenomena that can take place throughout the entire sample. Coalescence and flocculation phenomena both lead to an increase

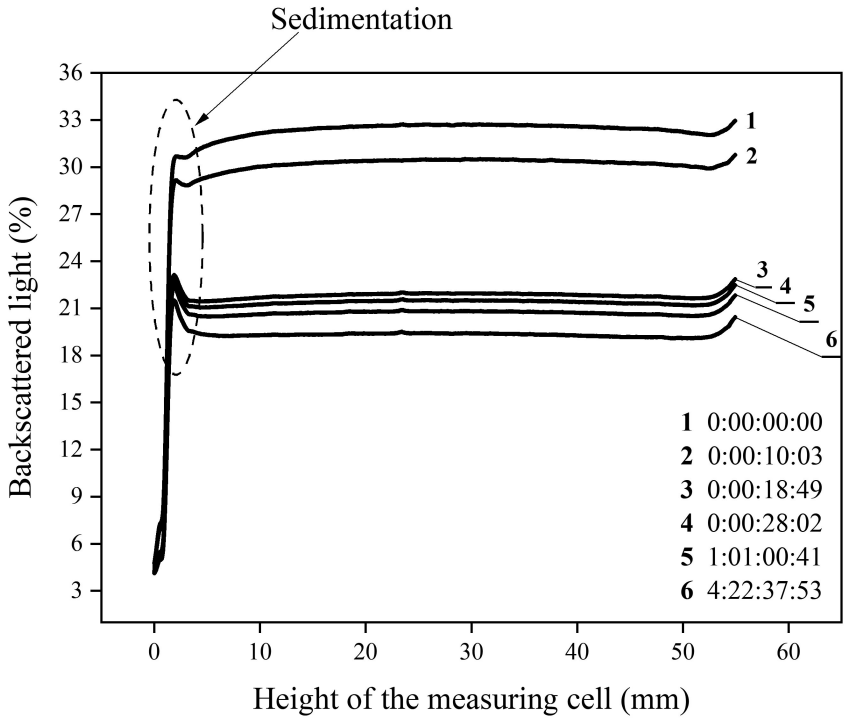

Fig. 5. Backscattered light intensity as a function of the height of a measuring cell and time for EM content of 15% (w/w)

in particle size. The difference between these two phenomena is that coalescence is irreversible and leads to the amalgamation of interfaces, resulting in a single drop, while flocculation is only particle aggregation (Jódar-Reyes et al., 2010). Flocculation can be also irreversible, and in that case it is called coagulation. In some cases, flocculation can lead to coalescence. The increase of backscattering throughout the whole vial is possible due to the formation of aggregates via coalescence or flocculation. Therefore, coalescence and flocculation are the phenomena that probably occur in the analyzed emulsion with a larger amount of emulsifier. However, it should be observed that the addition of emulsifier in the quantity of 15% (w/w) not only lowers the scope of BS intensity values, but also causes a slight increase of backscattered light intensity at the bottom of the sample. This phenomenon will affect the volume fraction of particles in the sample. Thus, the emulsion destabilization showed in Fig. 5 results from particle aggregation leading to particle migration, especially sedimentation.

Thus, it should be stressed that in the system of EM content equal to 5% and 15% (w/w) destabilization process occurred in contrast to dispersion with EM content of 10% (w/w). It can be revealed both by the average volume diameters determined on the basis of drop size distribution (Fig. 6) and dependence presenting the effect of revolution frequency on drop size of dispersed phase in the emulsion (Fig. 7).

It should be mentioned that particle size is a key factor that affects emulsion properties such as emulsion viscosity (Heldmann et al., 1999) and stability (Ghosh and Rousseau, 2009). Particle size mainly depends on the processing conditions (Walstra, 1993), especially shearing intensity and/or stirring rate during the emulsification process (Rosdi et al., 2018). The smaller particle size, the less likely destabilization of emulsions through sedimentation (Degner et al., 2014).

Thus, the emulsion with EM content of 10% (w/w) is the most stable one. It has been confirmed by the mean value of volume drop diameter in Emulsion 2 (d = 1.03 μm) – which in the most stable system should assume the lowest value (Zalewska et al., 2017) – and the decrease of drop diameter from the value of 10 μm for 2×10^3 rpm to the value of 0.5 μm for 15×10^3 rpm. It justifies the increase of dispersion system homogeneity and, at the same time, stability with the increasing frequency of revolutions.

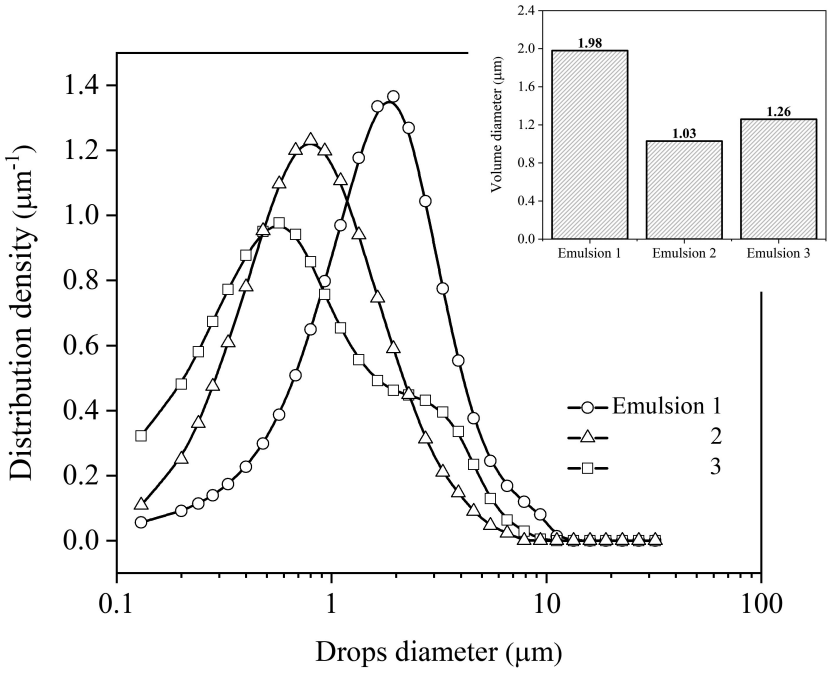

Fig. 6. Drop size distribution in emulsions and volume mean diameter as a function of EM contents

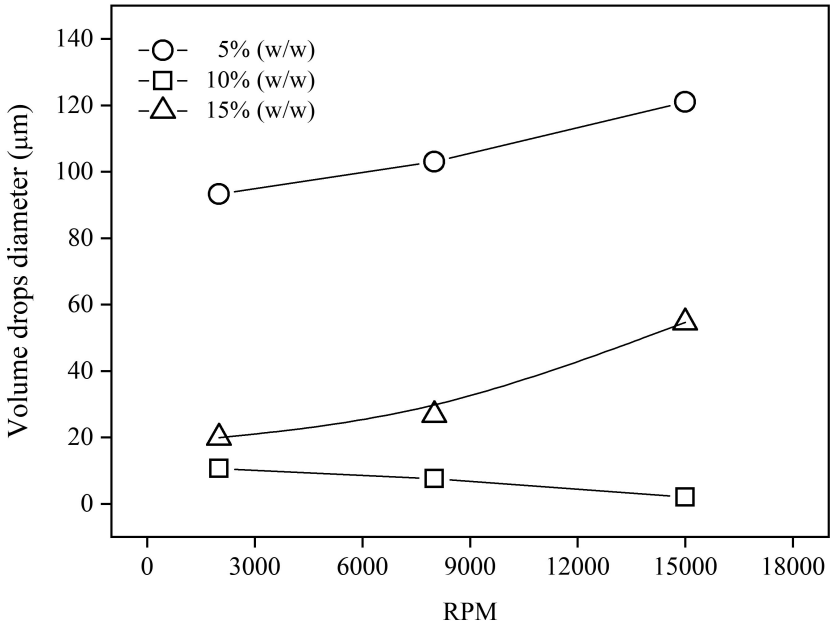

Fig. 7. Volume mean diameter of dispersed phase as a function of RPM and EM contents. The dependence was made using TLab-THERMO serving as a software of Turbiscan LAB optical analyzer

The addition of emulsifier in the amount of 5% and 15% (w/w) generated in the system drops of the average diameter of about 2 μm and 1.26 μm (Fig. 6), as well as a drop of system homogeneity manifested by less visible regularity and consisting in the increase of drop diameter with the increase of revolution frequency (Fig. 7).

The microscope images of the tested emulsions presented in Fig. 8 confirm the conducted assessments. They verify the occurring structure and particle distribution in the system. In case of Emulsions 1 and 3 with the EM content of 5% and 15% (w/w), respectively, the heterogeneous microstructure with marked spaces

filled with continuous phase between the drops has been clearly visualized. Emulsion 2 with the EM content of 10% (w/w) presents a system of dispersed phase drops of smaller size and uniform arrangement in the whole sample volume, which confirms increased stability of Emulsion 2, compared with the emulsions marked as 2 and 3 (Fig. 8).

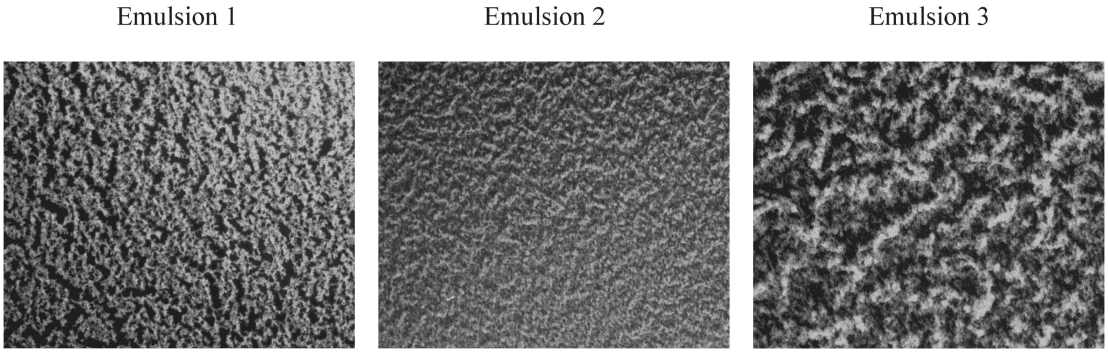

Fig. 8. Optical micrographs of emulsions 1, 2 and 3 at a magnification of 2 × 0.06

4. CONCLUSIONS

This paper concerns the selection of quantity of emulsifier (Polysorbate 80) to improve stability of plant origin emulsion. The analyzed emulsion is used to supplement animal diet in winter season to support their immune system. The addition of emulsifier to dispersion system in the quantity of 5% to 15% (w/w) was analyzed.

From the present analysis the following conclusions may be drawn which may be helpful in a preliminary assessment dealing with the selection of technological conditions:

- Turbiscan Stability Index values decrease with increasing amount of emulsifiers, and – in case of the analyzed dispersion – the added amounts are not sufficient to consider the system as a stable one.
- The addition of emulsifier in the quantity of 5% (w/w) and 15% (w/w) causes emulsion destabilization by migration of dispersed phase, the difference being that 5% (w/w) addition leads to creaming while 15% (w/w) addition causes sedimentation. Thus, there exists the amount of emulsifier for which the system can be considered to be stable.
- The most stable system was the emulsion containing 10% (w/w) of emulsifier and homogenized for at least 13 min. For a determined amount of emulsifier the volume drop diameter takes the lowest value and decreases with increasing frequency of revolutions. For the remaining amount of emulsifier the average value of volume drop diameter increases, but for lesser amount of emulsifier you can expect larger drop size.
- The analysis carried out in this study not only enriched the knowledge on the effect of emulsifier content on the performance of plant origin dispersion systems but also makes us aware of the fact that determination of the optimum emulsifier quantity is not an easy task from the technological viewpoint. The obtained results can be used to avoid complicated experimental tests prior to the application of the emulsions in industrial conditions.

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