Development of the emulsions containing modified fats formed via enzymatic interesterification catalyzed by specific lipase with various amount of water

RUNNING HEAD

Emulsions based on enzymatic modified fats

DEVELOPMENT OF THE EMULSIONS CONTAINING MODIFIED FATS FORMED VIA ENZYMATIC INTERESTERIFICATION CATALYZED BY SPECIFIC LIPASE WITH VARIOUS AMOUNT OF WATER

Małgorzata Kowalska[1], Magdalena Woźniak[2,3], Anna Krzton-Maziopa[4], Serge Tavernier[3], Łukasz Pazdur[3], Anna Żbikowska[5]

[1]Faculty of Material Science, Technology and Design, Kazimierz Pulaski University of Technology and Humanities, 27 Chrobrego St, 26-600 Radom, Poland

[2]Faculty of Economic and Legal Sciences, Kazimierz Pulaski University of Technology and Humanities, 33 Chrobrego St, 26-600 Radom, Poland

[3]Faculty of Applied Engineering, University of Antwerp, Salesianenlaan 90, 2660 Hoboken, Belgium

[4]Faculty of Chemistry, Warsaw University of Technology, Noakowskiego 3,00-664 Warsaw, Poland

[5]Faculty of Food Sciences, Warsaw University of Life Sciences – SGGW, Nowoursynowska 159C, 02-787 Warsaw, Poland,

*Corresponding author

mkowalska7@vp.pl

tel. 48 48 3617547

Abstract

The aim of this work was to obtain and evaluate the stability of new emulsion systems, in which diacylglycerols derived from enzymatic interesterification of mutton tallow with hemp oil were used as emulsifiers. In order to achieve a higher content of the polar fat fraction in the final fat blend, a different amount of water was added to the reaction mixtures. The modified fats with a mixture of mono and diacylglycerols served as a fat base for emulsions. Based on the results of Turbiscan test, the droplet size, emulsion texture, and studies of rheological properties, it was found that addition of water to the reaction mixture in the range of 1.00 - 1.25%, wt./wt., caused the formation of a sufficient amount of emulsifiers stabilizing the dispersion system.

The novelty of this work was to determine the optimal amount of water added to the interesterification of mutton tallow with vegetable oil, ensuring the synthesis of a high-efficiency emulsifying system. Another new aspect of this work was to show that the diacylglycerols obtained during such fat modification constitute effective emulsifiers for new stable emulsion systems that may find potential use as food emulsions (dressings) or cosmetic products dedicated to sensitive skin.

Key words: interesterification, diacylglycerols, lipase, emulsion, turbiscan stability index

Introduction

Emulsion stability is the term which describes the resistance to changes in its physicochemical properties overtime. It depends on several parameters, including formulation variables (nature and amount of stabilizing agent, e.g. thickener, viscosity of the continuous phase, density difference between continuous and dispersed phases) [1]. However, the surface-active components play the most important role in stabilizing an emulsion system. In the last years, an intensive achievement was done on the development of biodegradable compounds presenting emulsion stabilizing abilities. This group of substances includes compounds constituting the polar fraction of fats (mono and diacylglicerols) [2].

Mixtures of monoacylglycerols (MAG) and diacylglycerols (DAG) are widely-known and frequently used as emulsifiers in industrial production of functional fats [3]. Currently, there are more and more publications in which the properties and confirmed applications of these compounds are described [4, 5]. Nowadays, there are several methods available for the synthesis of DAG. Generally, DAG are enzymatically produced by direct esterification, glycerolysis, interesterification, partial hydrolysis, or the combination of partial hydrolysis and interesterification [6].In general, however, the proposal to use these compounds results from the addition of these compounds to the dispersion system as separate substances. This work demonstrates the possibility of producing fat and at the same time a suitable amount of mono and diacylglycerols that is able to stabilize the emulsion system.

Recently, interesterification processes attracted much attention as an alternative methods to modify the physicochemical properties of fats in the food industry [7]. As a result of interesterification, fatty acids (FA) are redistributed within a structure of

triacylglycerol molecule, or between triacylglycerols. The process of interesterification, in contrast to the commonly used hydrogenation, does not change the composition of fatty acids, so that the product does not contain *trans* FA [8]. It is well known that trans FA adversely affects human health, resulting in, inter alia, an increased risk of heart disease [9]. In this context, many European countries have banned the use of fats containing trans isomers in food products [8].

Due to the modification of triacylglycerols structure, interesterification affects physical properties of fats such as crystallization behavior, plasticity, melting point, solid fat content and texture [10]. Therefore, it is commonly used for producing new and more valuable fats with improved functional properties, to meet specific nutritional and functional requirements of food industry [8].

Generally, interesterification can be catalysed both enzymatically and chemically. Enzymatic catalysts are obtained from microorganisms such as bacteria, yeasts and fungi such as Candida Rugosa or Mucor miehei. Their most important advantage is regioselectivity targeting of the FA to the specific position in the triacylglycerol molecule [11]. Water content in enzyme preparations used as reaction biocatalysts is one of the most important factor influencing the reaction [12]. During interesterification a hydrolysis of fats occurs. Additional amount of water added to the reaction system causes imbalance between the interesterification process and the hydrolysis of the fat. Therefore, interesterified fats contain increased amount of polar fraction (DAG, MAG and FFA). Mono and diacylglycerols present in the modified fat blend obtained by this process can be used as emulsifiers in dispersion systems.

However, the management of the industrial application of emulsions containing interesterified fats requires a broader understanding of their physicochemical and functional properties as well as their stability during the storage process. The aim of this study was to obtain and evaluate the properties and stability of emulsions containing emulsifiers present in interesterified fats. Novelty of this work is the determination of the optimal amount of needed water in the enzymatic reaction environment, which can activate lipase most effectively, and also proving that there is a possibility to form novel emulsions based on diacylglycerols formed during fat modification process.

MATERIALS

The following materials were used for experimental work: Hemp Oil (Oleofarm); Mutton Tallow (donated from Meat-Farm Radosław Łuczak, Stefanowo, Poland); Lipase, immobilized on immobead 150 from *Rhizomucor Miehei*, ≥ 300 U/g (Sigma Aldrich). Hydranal Solvent and Hydranal Titrant were obtained from Honeywell Fluka. Sunflower lecithin (RF Solutions), carboxymethylcellulose (Pronicel Sp. z o.o.). Sodium benzoate, potassium hydroxide, alcohol solution of phenolphtaleine, ethyl alcohol and diethyl ether were obtained from POCh. THF, heptane, methanol, petroleum ether and silica gel were obtained from Acros Organics, sodium hydroxide and ethyl acetate (Fisher Scientific) were also used.

METHODS

Determination of water content in Lipase

Water content in a catalyst (Lipase from *Rhizomucor Miehei*) was carried out by means of Karl-Fischer titration using KF Titrando (Metrohm, Filderstadt, Germany).

Sample was dissolved in a working medium – the mixture of methanol, imidazole and sulphur dioxide (Hydranal Solvent) and was titrated with mixture of methanol and iodine (Hydranal Titrant-5). The measurement was performed in triplicate.

Synthesis of diacylglycerols

Synthetic diacylglycerols have been obtained from monoacylglycerols. The reaction was performed using acid-catalyzed solvent-assisted conditions at low temperature. Synthesized diacylglycerols were purified by column chromatography filled with silica gel. The detailed procedure for the synthesis and purification of DAG is described in the publication [13].

Procedures and determinations of FATS

Determinations were performed for raw fats (Hemp Oil and Mutton Tallow), non-interesterified blend (NIE) - blend before the reaction and interesterified blends (EIE) - after the modification.

Bleaching of mutton tallow

Before the interesterification, mutton tallow was bleached and deodorized. For this purpose, the product was melted, then the bleaching earth was added in the amount of 2% (wt./wt.) in relation to the fat mass. The tallow was heated under reflux at 80 ° C for 1 h. After this time, the sorbent was filtered on a paper filter by hot filtration at 70 ° C.

Enzymatic interesterification (EIE) reaction

Mutton tallow and hemp oil were mixed in a ratio of 1: 1 (wt./wt.). The prepared mixture was divided into 9 samples of the same volume. Next the flasks containing

the prepared mixtures were placed in a shaker equipped with a water bath (SWB 22N, Labo Play, Poland) and thermostated at a reaction temperature of 60 ° C for 15 minutes. After this time, an enzyme catalyst was added in an amount of 5% relative to the fat mass. Then a different addition of distilled water was introduced into the flasks (denoted as F1 - F8, see Table 1): 10, 11, 12, 13, 14, 15, 20, 25% wt./wt., relative to the enzyme mass. The samples were shaken vigorously for 6 hours. Reactions were stopped by hot filtering the enzyme from the reaction mixture on a Büchner funnel with a paper filter. In parallel, a reference sample (F0) containing non-interesterified blend of fats was prepared the same way, without addition of enzyme and water.

Determination of fatty acid composition

Fatty acid (FA) composition of raw fats and fat blends (NIE and IE) was determined using gas chromatography-flame ionization detector (GC-FID) after conversion of the fats to fatty acid methyl esters (FAMEs) via esterification reaction. The analysis was performed using the Agilent Technologies 6890N GC System equipped with a EC-Wax capillary column (Alltech, 30 m × 0.25 mm × 0.25 µm). The oven temperature was increased from 50 to 180 °C (10 °C/min) and then to 240 °C (5 °C/min). The detector and injection temperature were maintained at 300 and 250 °C, respectively. The injection volume was 1.0 µL. Splitless mode was used and lauric acid methyl ester was used as an internal standard. The measurements were performed in triplicate.

Acylglycerol separation

Acylglycerol separation (MAG, DAG and TAG) was performed on a F8 fat sample, using column chromatography, with a silica gel filled column as the stationary phase

and mixtures of petrol ether and ethyl acetate in various proportions as eluent. The sample F8 was selected for the determination because it contained the largest amount of polar fraction. An analysis was carried out for the F8 sample as representative, in order to check whether the profile of fatty acids acid changed after the interesterification process.

MAG, DAG and TAG content

The amount of MAG, DAG and TAG in fat and fat blends was determined using gel permeation chromatography (GPC) by Agilent 1100 series GPC system. Isocratic elution with tetrahydrofuran (stabilized with BHT) was used on a Phenogel column (Phenomenex, 300 × 7.80 mm, 5 micron, 100 Å). Refractive index detector was used. THF was used as a sample solvent. The measurements were carried out in triplicate and calculations were made on a basis of calibration curves.

Slip melting point

The slip melting point (SMP) was determined according to ISO method [14] with open capillary tube method. The results were presented as a mean value of 3 determinations.

Acid value

The acid value (AV) was determined according to ISO method [15]. The presented results are calculated as a mean value of 3 determinations. Samples were dissolved in a mixture containing equivalent amounts (v/v) of ethanol and diethyl ether, then were titrated with 0.1 M aqueous solution of potassium hydroxide using phenolphthalein as an indicator. The content of FFA (%FFA) was estimated on the basis of the acid value, according to the following equation:

$$\% \text{ FFA} = \frac{(v - v_b) \times N \times 28.2}{w}$$

where: v is the volume [mL] of titration solution, v_b is the volume [mL] of the blank, N is the normality of the 0.1M KOH, w is the weight of the sample of fat [g], 28.2 is the molecular weight of oleic acid divided by 10.

Optical microscopy

Microscope images of non-interesterified and interesterified fat blends were observed and photographed using a Delta Optical Genetic Pro Trino microscope, a DLT-Cam PRO digital camera at ×10 and ×40 objective and polarization equipment Genetic Pro #DO-4805 (Delta Optical, Poland). Fat blends were melted and approximately 10 µL of sample was placed on a glass slide and covered with a glass slip. The prepared samples were heated at 70ºC for 10 minutes and rapidly cooled to ambient temperature. The microphotographs were taken after tempering of the samples at ambient temperature for 24h. The procedure was adopted from the publication [16].

Procedures and determinations of Emulsions

Emulsions formulation

Aqueous phase of each emulsion was prepared by dispersing of 1.0 % (wt./wt.) carboxymethylcellulose (CMC) in distilled water over 1 minute. An oil phase of emulsions E1-E8 consisted of 30.0 % (wt./wt.) of interesterified fat (Table 2). The oil phases of emulsions E9 and E10 were prepared by mixing of 30.0 % (wt./wt.) of non-interesterified fat blend and suitable amount and type of emulsifier. The exact compositions are listed in Table 2.

The emulsions were prepared by adding the aqueous phase to the oil phase, both phases were pre-heated to 50-55 °C. Homogenization of the phases was achieved with a T18 digital ULTRA-TURRAX homogenizer equipped with S18G-19G dispersing head (IKA, China) for 4 minutes. Afterwards, emulsions were cooled to room temperature and 0.3 % (wt./wt.) of preservative (sodium benzoate) was added. Total mass of each emulsion was 100.0 g.

pH determination

The pH of the dispersions was determined at ambient temperature using SevenMulti pH meter (Mettler Toledo, Switzerland); precision ± 0.01; equipped with a pH electrode (InLab Expert Pro, Mettler Toledo, Switzerland). The buffer solutions for standardization were obtained from Mettler Toledo at pH 4.01 and 7.00. The measurements were taken on emulsions stored over 30 days at 5°C. pH determinations were performed in triplicate for each emulsion.

Texture analysis

Texture evaluation was obtained by consistency analysis using a Texture Analyzer CT3 (Brookfield Engineering Laboratories, Inc., Middleboro, MA, USA) equipped with Brookfield Texture Pro CT software. One-cycle compression and nylon spherical shaped probe (TA43; diameter 25.4 mm) were used. All samples were measured in the same container type (cylindrical shape: 50 mm depth; 30 mm internal diameter). The test and return speed was 0.5 mm/s, pre-test speed 2 mm/s, with target depth of 10 mm, trigger load was 1 g, data rate 10 points/s The measurements were carried out at room temperature. The measurements were taken on freshly prepared and stored emulsions (30 days, 5°C), in triplicate.

Emulsions destabilization

The kinetics of destabilization occurring in the prepared emulsions was determined using Turbiscan Lab (Formulation, France). The measurement technique uses multiple light scattering analysis, more specific - pulsed near-IR light source at wavelength 880 nm and two synchronized detectors (collecting transmitted (T) and backscattered (BS) light) . The principle of the determination is based on variation of the BS and T signals, caused by changes in the droplet volume fraction migration or mean size change due to coalescence [17]. The samples were placed in cylindrical measuring cells with a flat bottom. During the measurement, they were scanned with a beam of light every 40 μm at a temperature of 25 °C. Samples were stored at 30 ºC and their analysis was performed during one month, in 2-4 days intervals.

The data obtained are presented as variation of back-scattering profiles (ΔBS) and Turbiscan Stability Index (TSI). BS was calculated on a basis on the following equation:

$$\mathrm{BS} = \frac{1}{\sqrt{\lambda^*}}$$

where: λ* was the photon transport mean free path in the sample, which was defined as:

$$\lambda^*(\Phi, \mathrm{d}) = \frac{2\mathrm{d}}{3\Phi(1-\mathrm{g})\mathrm{Q_s}}$$

where Φ is the volume fraction of dispersion particles, d is the mean diameter of dispersion particles and g and Q_S are the optical parameters given by the Mie theory [18]. The obtained BS data were presented as BS variations (ΔBS), where each of the subsequent measurements was subtracted from the first one in order to more easily visualize the changes taking place in the samples.

TSI was calculated according to BS changes that indicate the particles aggregation and dynamic migration [19], according to the following formula:

$$TSI = \sum_{i} \frac{\Sigma_h |scan_i(h) - scan_{i-1}(h)|}{H}$$

where: $scan_i(h)$ is average backscattering for each time (i) of measurement, $scan_{i-1}(h)$ is average backscattering for the i-1 time of measurement, and H is the sample height [20]. The higher is the TSI, the stronger is the destabilization in the sample.

All above calculations were performed using TurbiSoft 2.0. software.

Dynamic viscosity

The viscosity of the prepared emulsions was determined using Brookfield DV-III Ultra rheometer, model HA with helipath spindle set (Brookfield Engineering laboratories, USA), using T-bar spindle no. 93 (T-C) at 10 rpm. The measurements were performed at ambient temperature.

Viscoelastic behavior and rheological stability tests

Viscoelastic behavior of the investigated materials was studied with a rotational rheometer Physica MCR301 equipped with an air bearing and Peltier device for precise temperature control. Measurements were performed in an oscillatory mode using measuring cell of parallel plate geometry (50mm diameter, 1mm gap). For determination of viscoelastic properties of the prepared emulsions oscillatory tests at constant angular frequency (1 rad/s) and variable deformation amplitude (0.1 - 100 %) were carried out. Long term stability of the prepared materials and the microstructure formation was analyzed using frequency sweep tests conducted at

constant deformation amplitude of 0.1% at angular frequencies within the range 10^{-1} -100 rad/s. Rheological data were analyzed with a Rheoplus software.

RESULTS AND DISCUSSION

FATS

Fatty acids composition

Results collected in Table 3, revealed that the change of FA compositions before and after enzymatic interesterification was insignificant, which is also in accordance with the report of Chen et al. [21]. Analyzing the content of fatty acids in the tested samples, it was observed that the main acids present in hard fat (mutton tallow) were: palmitic, stearic and oleic acids. Saturated acids constituted about 65% of all acids, although this is lower than in the popular beef tallow [22]. On the other hand, in the case of hemp oil, mainly linoleic, α-linoleic and oleic acids were found, which constituted as much as 84% of all acids present in this oil. The contents of these acids were consistent with the results obtained by other researchers [23, 24]. Considering the composition of fatty acids in the TAG and DAG fractions, it was found that the amounts of individual acids in these fractions were similar. Due to the high reactivity, the MAG fraction was characterized by very low values (content) of individual acids (practically it was invisible on chromatograms), therefore it was not included in the Table 3.

Fat blends composition

Analysing the results concerning the determination of the polar and non-polar fractions for the original products, ie mutton tallow and hemp oil, one can observe the predominant share of the triacylglycerol fraction (over 95%) in both raw

materials. The presence of monoacylglycerols in both substrates is not visible, but both materials contain a small proportion of dialcylglycerols, which is slightly higher in hard fat (Table 4).

After the interesterification process, a decrease in the content of the non-polar fraction was observed in the fatty blends, with the simultaneous increase in the content of the polar fraction. Similar results were also obtained by Zainal et al. [25] who indicated that the interesterification process changed the composition of acylglycerols and the content of FFA in fat blends, due to the partial hydrolysis occurring there. Moreover, it was observed that with the addition of water to the enzyme preparation during the interesterification of fatty mixtures, the amount of DAG, MAG and FFA in the modified mixture increased. Due to the high reactivity of monoacylglycerol, an insignificant amount of these compounds was observed in post-reaction mixture. The calculated content of the emulsifier fraction as the sum of MAG and DAG, which was used as an emulsifier, is presented in Table 4. Similar results confirming the growth of the polar fraction after the interesterification process were also obtained by Gruczynska et.al. [26], who carried out enzymatic transesterification of lard and rapeseed oil mixtures in proportion 1:1 wt./wt., using the immobilized Lipase from *Rhizomucor miehei*.

In general, in each case after interesterfication, the FFA content was higher than originally obtained for the physical mixture (Table 4). The acid value and the associated content of free fatty acids is one of the proofs confirming the interesterification process, and in our case also the simultaneous hydrolysis [27].

In this work, obtaining a polar fraction was an intentional and desirable effect as mono and dicylglycerols are stabilizers of dispersion systems [28]. In addition, these

components act prooxidatively, which allows to increase the stability of fat [26]. On the other hand, the formation of free fatty acids that occurs in parallel with MAG and DAG during interesterification, may be beneficial in cosmetic preparations [29]. Due to the addition of water to EIE reaction, partial fat hydrolysis was forced [27]. The ratio of polar and non-polar fraction in the interesterified blends was significantly dependent on the water content in the reaction system [27].

Due to positional specificity of Lipase from *Rhizomucor miehei* (sn-1,3), fat hydrolysis occurred mainly in external positions, i.e. sn-1,3 [30]. According to Ribeiro et al. [31] in vegetable fatty triacylglycerols, saturated fatty acids are almost exclusively in the sn-1 and sn-3 positions, while the unsaturated ones are in the sn-2 position. Thus, if a regiospecific enzyme preparation is used, such as in the present work, the proportion of individual acids (saturated and unsaturated) in the specified positions should not change. The opposite conclusions can be drawn in case of accidental distribution (eg in chemical interesterification), which results in an increase in the content of saturated acids in the central triacylglycerol positions and an increase in the contribution of unsaturated acids in external positions [31].

Slip melting point (SMP)

The melting point of fats is a very important parameter, which constitutes a basis for the description and development of fats [31]. Changing the content of individual acylglycerols and redistribution of fatty acid residues in molecules during the EIE reaction causes changes in SMP fats [32].

The presented results show a decrease in SMP in all interesterified samples, compared to the mixture before the reaction (Table 4). The reduction of SMP values for samples after the reaction indicated that the reaction had achieved sufficient yield

and changes in the physical properties of product appeared [33]. In the case of interesterified mixtures, the lowest SMP value was obtained for the F8, while the highest for the F1 sample, these values were 22.2 and 38.5 ºC, respectively (Table 4). Similar decrease of SMP after EIE was obtained by Goli et al. [33], who analyzed changes in this parameter in enzymatically interesterified CLA-containing fats using *Candida antarctica* lipase.

Optical microscopy of fats

The kinetics of fat crystallisation affect their structure that is also closely related to rheological properties and plasticity of fats. Due to changes in the structure of triacylglycerols occurring during the interesterification process, the rate of crystal formation and their dimensions change significantly [34]. Figure 1 shows the microstructure of the fat blends before and after enzymatic interesterification analyzed by polarized light microscopy (PLM). The crystal structure of non-interesterified blend (F0), shown in Figure 1, consists of asymmetric spherulites surrounded by long needles of radial alignment. After interesterification reaction the visible changes in the structure of the interesterified fat blends are visible. The spherulite shapes become more symmetrical, and also agglomerates of very small crystals with regular round shapes were formed. The appearance of spherulite also changed as they grew to be more dense in the halo-region. Similar changes in the spherulite morphology after interesterification were also seen by Rousseau et al. in palm oil/soybean oil and lard/canola oil blends [35].

Previous studies on interesterified fats [36] indicated that changes in fat crystallization have a significant impact on sensory sensations, such as mouth feel and spreadability, and thus on the overall sensory evaluation of fat products.

Therefore, studies on fat crystallisation, combined with sensory evaluation, may be useful in determining the areas of final application of interesterified fats, both in the food, cosmetic and even pharmaceutical industries, where sensory attributes are very important.

EMULSIONS

pH determinations

Emulsion stability is strongly associated with pH [37]. According to authors [38, 39] the addition of the chosen thickeners can influences on the increase or decrease of the final emulsion pH which may result in reduced its stability. Figure 2 shows the pH value of freshly prepared emulsions. The pH of the prepared emulsions was within a range of 6.2 - 6.9, which falls in the rage of human skin. The lowest pH value was obtained for E1, which contained interesterified fat F1. However, the highest pH value was obtained for the emulsion system E10, containing the physical mixture of fats and synthetic diacylglycerols obtained as a result of the synthesis from monoacylglycerols. The one of possible reasons for such a higher pH value in the E10 sample may be the presence of a salt derived from the emulsifier synthesis, which hydrolyzes in the alkaline environment.

Texture analysis

The texture of the emulsion is one of the parameters defining the functional properties of the product. The assessment of this parameter determines the fulfillment of specific requirements or acceptance of the tested product by the consumer. The following features such as: consistency, firmness, spreadability, stickiness [40] will affect the overall texture of the emulsion. Although the texture

assessment is included in the sensory evaluation, the result obtained from the designation is the sum of the components of the above parameters determined by different units, not as in the traditional sensory evaluation, where the assessment is based on a particular scale from 1 to 5. Texture analysis of the investigated products was performed for freshly prepared formulations stored at 5 °C over 30 days.

Analyzing the evaluated products, it was observed that the fresh emulsions E1-E8 were characterized by significantly lower "firmness" (up to 10 g) while the emulsions E9 and E10 showed values of 34 and 51 g, respectively (Figure 3). After 30 days of storage for almost all emulsions an increase in firmness was observed. The highest increase in this parameter was found in emulsions E7 and E8 (for E7, the increase was 36 g for E8 - 39 g). Smaller increments in the range up to 18 g were noted for the remaining samples containing diacylglycerols formed during interesterification. In contrast, the emulsion E9 with lecithin was characterized by the highest firmness of all samples taken into account during the 30-day storage period. The reverse observation was noted for E10, which after the said storage time needed less force to deform it than immediately after its preparation.

Another component of the emulsion texture assessed in the current work was spreadability. The values obtained are presented in Figure 3. According to the sensory definition, this parameter represents the work necessary to overcome the strength of internal bonds in a formulation [41]. According to Brookfield application notes for moisturizing creams a higher firmness coupled with the higher hardness indicate a less spreadable sample [42]. Yadav et al. [40], also stated that the final acceptance of the product depends on its spreadability. Considering the obtained spreadability results, in the evaluation immediately after preparation, samples E9 and E10 would be the least accepted. These results are consistent with the highest

viscosity values obtained for these samples. It should be noted, however, that the spreadability of E1-E9 products has deteriorated over time. However, when comparing these data with commercial formulations assessed by Yadav et. al [40], the obtained results for emulsions containing interesterified fat remain at a lower level.

It is generally assumed that adhesiveness of the sample represents its stickiness. This parameter is defined as the maximum force required to overcome the attractive force between a sample and any other surface [40, 43]. Analyzing the results of the mentioned parameter, it was observed that after 30 days of storage, almost all emulsions (E1-E9) required greater force in the adhesion test, compared to freshly prepared emulsions. The highest growth of this force, and at the same time the highest value of adhesiveness was found for emulsions E7 and E8, in which the increments were 16 and 17 g, respectively. The only emulsion in which the decrease in adhesiveness was observed after 30 days of storage was emulsion E10 (10 g). In general, the highest strength determined in the adhesiveness test for the tested samples, after 30 days of storage, was noted for the emulsion stabilized with lecithin.

Turbiscan

The Turbiscan Lab allows to determine the stability of opaque and concentrated dispersion systems, without dilution, and to detect destabilization phenomena much earlier than is generally possible with the naked eye. It can provide an information about the variations of back scattering (BS) profiles before the macroscopic physical change occurs in the dispersion system [18]. Thus, it allows to determine at the same time the type of destabilization taking place in the analyzed samples, i.e. creaming, sedimentation, coagulation, flocculation or Ostwald ripening. Beside

according to authors [44] Turbiscan lab is a suitable technique to study the separation of oil-in-water with good reproducibility.

The delta back-scattering profiles of prepared emulsions are presented in Figure 4. Analyzing these profiles, it was found that the differences in ΔBS values for emulsions E1-E4 are above 5% in the entire measurement period. Significant changes were observed for emulsion E5. For all of the E1-E5 emulsions, a progressive decrease of ΔBS was observed after about 10 days from the preparation. According to the Figure 4 the analyzed emulsions alter significantly with time due to the changes in the type of coalescence resulting most probably from merging of drops of the dispersed phase into larger agglomerates [17]. The final result of the progressive processes was the separation of the emulsion phases and thus the complete separation of the aqueous layer (collected at the bottom of the cell) from the fat phase remaining on the top of the vessel.

Analyzing the changes occurring for emulsion E6, a decrease in the rate of destabilization of the system was observed. After 25 days, changes in the intensity of backscattered light were slightly above 2%. Nevertheless, emulsion E6 broke down and a slight separation was observed after complete time of storage. Considering the evaluation of this emulsion, it was assumed that the content of emulsifiers in this system was close to a limit concentration of DAG allowing stabilization of the system in terms of durability. At the same time, it allows to conclude that emulsions E1-E6 were prepared with fats containing an insufficient amount of emulsifiers.

In case of further emulsion E7 and E8 ΔBS values throughout the measurement period were in the range of 1.7% and 2.0%, respectively. According to Celia et al. [18] no variation of dispersion droplets size occurs when the difference of back-

scattering light values is between the range of ±2%. Moreover, the difference higher than 10% indicates instability of the formulation. Taking into account the above information, as well as analyzing the ΔBS curve and assessing the appearance of the emulsion, it can be clearly stated that these systems showed no signs of destabilization (Figure 4). This means that the amount of emulsifiers produced during the EIE reaction was sufficient for emulsions stabilization.

To compare the stability of the presented emulsion systems, emulsion stabilized with a well-known and effective emulsifier – lecithin was evaluated [45]. The values of ΔBS determined for E9 were up to about 8% (Figure 4), which indicates a coalescence process that resulted in the destabilization of the emulsion [18]. However, it should be noted that visually no significant instabilities such as the phase separation seen in the E1 - E5 systems have been observed.

The emulsion E10, containing as a synthetic emulsifier DAG (Figure 4) was characterized by the least stability. The change in BS for this system for the entire storage period exceeded 15%. Interestingly, in the visual assessment, this sample looks quite stable, whereas according to the Turbiscan test, destabilization started already in the first days after emulsion formation. Destabilization in the analyzed sample detected with the Turbiscan test confirms, therefore, that the visual assessment of the stability of the emulsion is an insufficient criterion for assessing the change in the kinetics of the system [18].

In order to confirm the previously indicated information, the Turbiscan Stability Index, which provides an information about kinetics of coalescence in a function of sample shelf-life was determined. When analyzing the TSI values, it was observed that emulsions E6, E7 and E8 were characterized by the lowest value of the coefficient. It

is assumed that the low value of the coefficient and the smallest change in the value of this parameter during sample storage prove the stable nature of the system [46]. Considering the above, emulsions E1, E5 and E10 were the most unstable systems (Figure 5). They showed a high variation of TSI (the highest increase).

All the emulsions were obtained with good reproducibility and characterized by different droplet size, which was found in the range 3.4 - 14.8 μm (Table 5). The smallest droplet size was found in emulsions E7 and E8, for which the smallest particle size changes were also observed during the entire storage period. According to literature data, the small size of droplets and the smallest changes in particle size during storage, allow to qualify the emulsion as a stable system [1]. Considering this type of determination, both emulsions can be classified as systems with good homogeneity and stability. It was observed that the highest dispersion of particles, i.e. the greatest variation of the particle size during the entire emulsion storage period, had emulsion E10.

Dynamic viscosity

One of the key parameters responsible for the proper stability of the systems and sensory evaluation is its viscosity [47]. Analyzing the viscosity of the presented emulsion systems, it was observed that this parameter was at a comparable level for samples containing emulsifiers produced during interesterification. The value of this parameter after 24h was in the range from 1014 cP to 2033 cP for E3 and E7, respectively (Figure 6). For lecithin containing emulsion, the viscosity was higher after 24h and amounted to 5803 cP. The highest value of viscosity in the first 24 hours after preparation was observed for the sample containing the emulsified emulsifiers (DAG) (7312 cP). After one month of storage, the viscosity increased for

all emulsions tested. The highest values were determined for emulsions E7, E8 and E9. The smallest increase was observed for emulsion E10. The results obtained are similar to the viscosity of emulsion systems containing the same viscosity modifier - carboxymethylcellulose [48]. Hayati et al. also noticed that the viscosity of carboxymethylcellulose (CMC) containing emulsions increases over time. Analyzing the above information, it can be concluded that the best effects were obtained for systems with synergistic action of the emulsifier and the viscosity modifier (CMC), which allowed to obtain a higher viscosity and thus reduced the destabilizing changes. According to Stokes' law, by increasing the viscosity of the emulsion, the gravitational separation of the phases is delayed, which increases its stability [1].

Viscoelastic behavior and rheological stability tests

Oscillatory rheological tests are convenient method enabling evaluation of emulsion consistency and related properties such as the strength of the emulsion microstructure [49]. The oscillatory tests are performed at stationary or quasi-stationary conditions (in the range of small deformation of emulsion microstructure) and as concentrated emulsions are the strain-dependent network systems, the relatively small strains imposed to the microstructure may serve as the indication of microstructure deterioration. As the oscillatory tests reveal the microstructure rigidity and its resistance to intentionally applied deformations, they can also serve as the *in-situ* tests of emulsion stability in prolonged period of time, especially when these tests are performed in the wide frequency range [50]. The dependence of storage (G') and loss (G") moduli on the applied strain amplitude at constant angular frequency is shown in Figure 7. It is clearly seen that the rheological behavior of the investigated systems strongly depends on the composition of the investigated emulsions, and especially on the amount of polar fractions formed during

interesterification process. Emulsions with higher amounts of polar fractions (E6, E7, E8) show considerably broader linear viscoelastic range (LVR) as can be seen in Figure 8. The observed values of G' modulus are also slightly higher, which can be treated as a proof of the formation of microstructure of greater stability in these emulsions. Among all the investigated emulsions E7 and E8 exhibit the highest rigidity revealed by almost twice higher values of G' modulus in comparison to the other emulsions containing interesterified fats, although they are considerably smaller in comparison to the reference emulsion (E9) stabilized by lecithin. Emulsion E10 stabilized by synthetic DAG shows very high values of G' modulus, but quite narrow LVE range, which suggests that stability of this system is rather moderate, and the high values of storage modulus can result from formation of bigger aggregates or creaming that take place in this system.

In the strain-dependent emulsion systems, one should expect a higher dependence on frequency for the dynamic moduli and smaller differences between them. To study these relations we have performed the corresponding frequency tests and their results are shown in Figure 8. The frequency sweeps show clearly that all of the investigated emulsions are frequency dependent. The observed changes are rather moderate for the emulsions containing interesterified fat blends and the values of both moduli are comparable. However, when comparing the behavior of emulsions at high frequency and low regimes the systems with lower amounts of polar fractions (E1-E5), seem to be more frequency dependent as the slope of G' curves is more steep in comparison to their counterparts with higher polar fraction contents (E6-E8). These relative changes may give some indication to the enhanced stability of E6-E8 emulsion systems in a prolonged time period.

To compare the viscoelastic behavior of the investigated emulsions a damping factor (named also as loss tangent, tanδ) was used. The damping factor is calculated as ratio of G'' and G' modulus: tanδ=G''/G'. This parameter illustrates the strength of interactions that are present in the emulsion due to the formation of more rigid structure, and can be treated as the measure of its rigidity. In general the lower values of loss factor the more rigid is the material under investigation. According to the results shown in Figure 9 the strongest interactions appear in the reference emulsion stabilized with lecithin. For the systems containing interesterified fats one can see that emulsions with higher amounts of polar fractions (E6-E8) show also lower rigidity (higher values of tanδ). The oscillating value of damping factor at low frequency region observed for emulsions E1 and E2 and also for E9 and E10 may be the indication of destabilization of these emulsions with time due to droplets coalescence or creaming processes taking place in the investigated systems.

CONCLUSIONS

In the presented work, by enzymatic interesterification of mutton tallow with hemp oil (1: 1, wt./wt.) new fats were obtained, with an increased amount of polar fraction (MAG, DAG and FFA) and modified physicochemical properties (increase in softening temperature, improved crystalline structure). Emulsions made from interesterified fat mixtures, where the water content in the enzyme preparation was ≤ 0.75%, were characterized by low stability. The amount of emulsifiers produced during the reaction turned out to be insufficient to stabilize the dispersion system. In turn, emulsions made from fatty mixtures formed during interesterification, where the addition of water to the enzyme preparation was in the range of 1.00 - 1.25%, showed high stability, higher than the emulsion containing sunflower lecithin, or synthetically produced diacylglycerols. Emulsion systems based on enzymatically

interesterified fat blends containing diacylglycerols can be an alternative for cosmetic and food emulsions.

References

[1] McClements, D. J. Critical review of techniques and methodologies for characterization of emulsion stability. *Crit. Rev. Food Sci. Nutr.* **2007**, 47, 611-649. DOI: 10.1080/10408390701289292.

[2] Kowalska, D.; Gruczyńska, E.; Kowalska, M. The effect of enzymatic interesterification on the physico-chemical properties and thermo-oxidative stabilities of beef tallow stearin and rapeseed oil blends. *J. Therm. Anal. Calorim*. **2015**, 120, 507-517. DOI: 10.1007/s10973-014-3869-1.

[3] Zhang, Z.; Ma, X.; Huang, H.; Li, G.; Wang, Y. Enzymatic Production of Highly Unsaturated Monoacyglycerols and Diacylglycerols and Their Emulsifying Effects on the Storage Stability of a Palm Oil Based Shortening System. *J. Am. Oil Chem. Soc.* **2017**, *94*, 1175-1188. DOI: 10.1007/s11746-017-3023-x.

[4] Verstringe, S.; Danthine, S.; Blecker, C.; Dewettinck, K. Influence of a commercial monoacylglycerol on the crystallization mechanism of palm oil as compared to its pure constituents. *Food. Res. Int.* **2014**, *62*, 694-700. DOI: 0.1016/j.foodres.2014.04.049.

[5] Szelag, H.; Macierzanka, A. Synthesis of modified acylglycerol emulsifiers in the presence of Na, K and Zn fatty acid carboxylates. *Tenside. Surfact. Det.* **2001**, *38*, 377-382.

[6] Liu, N.; Wang, Y.; Zhao, Q.; Zhang, Q.; Zhao, M. Fast synthesis of 1, 3-DAG by Lecitase® Ultra-catalyzed esterification in solvent-free system. *Eur. J. Lipid Sci. Technol.* **2011**, 113, 973-979. DOI: 10.1002/ejlt.201000507.

[7] Xie, W.; Zang, X. Immobilized lipase on core–shell structured Fe3O4–MCM-41 nanocomposites as a magnetically recyclable biocatalyst for interesterification of soybean oil and lard. *Food Chem*. **2016**, 194, 1283-1292. DOI: 10.1016/j.foodchem.2015.09.009.

[8] Xie, W.; Zang, X. Lipase immobilized on ionic liquid-functionalized magnetic silica composites as a magnetic biocatalyst for production of trans-free plastic fats *Food Chem*. **2018**, 257, 15-22. DOI: 10.1016/j.foodchem.2018.03.010.

[9] Xie, W.; Zang, X. Covalent immobilization of lipase onto aminopropyl-functionalized hydroxyapatite-encapsulated-γ-Fe2O3 nanoparticles: A magnetic biocatalyst for interesterification of soybean oil. *Food Chem*. **2017**, 227, 397-403. DOI: 10.1016/j.foodchem.2017.01.082.

[10] Dian, N. L. H. M.; Sundram, K.; Idris, N. A. Effect of chemical interesterification on triacylglycerol and solid fat contents of palm stearin, sunflower oil and palm kernel olein blends. *Eur. J. Lipid Sci. Tech.* **2007**, 109, 147-156. DOI: 10.1002/ejlt.200600198.

[11] Berry, S. E. E. Triacylglycerol structure and interesterification of palmitic and stearic acid-rich fats: an overview and implications for cardiovascular disease. *Nutr. Res. Rev*. **2009**, 22, 3-17. DOI: 0.1017/S0954422409369267.

[12] Tarnowska, K.; Kostecka, M.; Piotrkowicz, A.; Łobacz, M.; Kowalski, B. Interesterification of goose fat and rapeseed oil mixture using Candida rugosa lipase immobilized in alginate beads. *Riv. Ital. Sostanze Gr.* **2013**, 40, 95-105.

[13] Pazdur, Ł.; Geuens, J.; Sels, H.; Tavernier, S. M. Low-Temperature Chemical Synthesis of High-Purity Diacylglycerols (DAG) from Monoacylglycerols (MAG). *Lipids*, **2015**, 50, 219-226. DOI: 10.1007/s11745-014-3980-x.

[14] ISO Method 6321: 2000. Animal and vegetable fats and oils — Determination of melting point in open capillary tubes (slip point). Geneva, Switzerland, 2000.

[15] ISO Method 660:2009. Animal and vegetable fats and oils — Determination of acid value and acidity. Geneva, Switzerland, 2009.

[16] Fauzi, S. H. M.; Rashid, N. A.; Omar, Z. Effects of chemical interesterification on the physicochemical, microstructural and thermal properties of palm stearin, palm kernel oil and soybean oil blends. *Food Chem*. **2013**, 137, 8-17. DOI: 10.1016/j.foodchem.2012.09.086.

[17] Liu, J.; Huang, X. F.; Lu, L. J.; Li, M. X.; Xu, J. C.; Deng, H. P. Turbiscan Lab® Expert analysis of the biological demulsification of a water-in-oil emulsion by two biodemulsifiers. *J. Hazard. Mater*. **2011**, 190, 214-221. DOI: 10.1016/j.jhazmat.2011.03.028.

[18] Bendjaballah, M.; Canselier, J. P.; Oumeddour, R. Optimization of oil-in-water emulsion stability: experimental design, multiple light scattering, and acoustic attenuation spectroscopy. *J. Dispersion Sci. Technol*. **2010**, 3, 1260-1272. DOI: 10.1080/01932690903224888.

[19] Raikos, V.; McDonagh, A.; Ranawana, V.; Duthie, G. Processed beetroot (Beta vulgaris L.) as a natural antioxidant in mayonnaise: Effects on physical stability, texture and sensory attributes. *Food Science and Human Wellness*, **2016**, 5, 191-198. DOI: 10.1016/j.fshw.2016.10.002.

[20] Xu, D.; Zhang, J.; Cao, Y.; Wang, J.; Xiao, J. Influence of microcrystalline cellulose on the microrheological property and freeze-thaw stability of soybean protein hydrolysate stabilized curcumin emulsion. *LWT- Food Sci. Technol*. **2016**, 66, 590-597. DOI: 10.1016/j.lwt.2015.11.002.

[21] Chen, Q.; Li, Y.; Fu, J.; Ma, X.; Teng, Y.; Wang, Y. Production of diacylglycerol-enriched oils by enzymatic interesterification and molecular distillation using soybean oil and distilled saturated monoacylglycerol. *Eur. J. Lipid. Sci. Tech*. **2017**, 119. DOI: 10.1002/ejlt.201600332.

[22] Kowalska, M.; Bekas, W.; Kowalska, D.; Lobacz, M.; Kowalski B. Modification of beef tallow stearin by chemical and enzymatic interesterification with rapeseed oil. *Am. J. Food Technol*. **2007**, 2, 521-528. DOI: 10.3923/ajft.2007.521.528

[23] Teh, S. S.; Birch, J. Physicochemical and quality characteristics of cold-pressed hemp, flax and canola seed oils. *J. Food. Compos. Anal*. **2013**, 30, 26-31. DOI: 10.1016/j.jfca.2013.01.004.

[24] Oomah, B. D.; Busson, M.; Godfrey, D. V.; Drover, J. C. Characteristics of hemp (Cannabis sativa L.) seed oil. *Food Chem*. **2002**, 76, 33-43. DOI: 10.1016/S0308-8146(01)00245-X.

[25] Zainal, Z.; Yusoff, M. S. A. Enzymatic interesterification of palm stearin and palm kernel olein. *J. Am. Oil Chem. Soc*. **1999**, 76, 1003-1008. DOI: 10.1007/s11746-999-0196-y.

[26] Gruczynska, E.; Kowalska, D.; Kozlowska, M.; Kowalska, M.; Kowalski, B. Enzymatic interesterification of a lard and rapeseed oil equal-weight blend. *J. Oleo Sci*. **2013**, 62, 187-193. DOI: 10.5650/jos.62.187.

[27] Kowalska, M.; Zbikowska, A.; Tarnowska, K. Stability of emulsions containing interesterified fats based on mutton tallow and walnut oil. *J. Am. Oil Chem. Soc*. **2015**, 92, 993-1002. DOI: 10.1007/s11746-015-2659-7.

[28] Feltes, M. M. C.; Villeneuve, P.; Baréa, B.; Barouh, N.; De Oliveira, J. V.; De Oliveira, D.; Ninow, J. L. Enzymatic production of monoacylglycerols (MAG) and

diacylglycerols (DAG) from fish oil in a solvent-free system. *J. Am. Oil Chem. Soc*. **2012**, 89, 1057-1065. DOI: 10.1007/s11746-011-1998-2.

[29] Kowalska, M.; Żbikowska A.; Szerling K. Application of modified fats by enzymatic interesterification in emulsions. *Ital. J. Food. Sci.* **2011**, 23, 136-144.

[30] Kowalski, B.; Tarnowska, K.; Gruczynska, E.; Bekas, W. Chemical and enzymatic interesterification of beef tallow and rapeseed oil blend with low content of tallow. *J. Oleo Sci.* **2004**, 53, 479-488. DOI: 10.5650/jos.53.479.

[31] Ribeiro, A. P. B.; Basso, R. C.; Grimaldi, R.; Gioielli, L. A.; Gonçalves, L. A. G. Instrumental methods for the evaluation of interesterified fats. *Food. Anal. Method*. **2009**, 2, 282-302. DOI: 10.1007/s12161-009-9073-4.

[32] Nichols, D. S.; Sanderson, K. In, Sikorski, Z.E., Kolakowska, A. (eds) *Chemical and functional properties of food lipids*. CRC Press:, New York, 2003.

[33] Goli, S. A. H.; Sahri, M. M.; Kadivar, M. Enzymatic interesterification of structured lipids containing conjugated linoleic acid with palm stearin for possible margarine production. *Eur. J. Lipid. Sci. Tech.* **2008**, 110, 1102-1108. DOI: 10.1002/ejlt.200800134.

[34] Narine, S. S.; Humphrey, K. L.; Bouzidi, L. Modification of the Avrami model for application to the kinetics of the melt crystallization of lipids. *J. Am. Oil Chem. Soc*. **2006**, 83, 913-921. DOI: 10.1007/s11746-006-5046-6.

[35] Rousseau, D.; Marangoni, A. G.; Jeffrey, K. R. The influence of chemical interesterification on the physicochemical properties of complex fat systems. 2. Morphology and polymorphism. *J. Am. Oil Chem. Soc*. **1998**, 75, 1833-1839. DOI: 10.1007/s11746-998-0339-6.

[36] Piska, I.; Zárubová, M.; Loužecký, T.; Karami, H.; Filip, V. Properties and crystallization of fat blends. *J. Food. Eng*. **2006**, 77, 433-438. DOI: 10.1016/j.jfoodeng.2005.07.010.

[37] Kowalska, M.; Zbikowska, A.; Marciniak-Lukasiak, K.; Smiechowski, K. Stability and the distribution of droplets in walnut oil water-based emulsions formed at different pH. *J. Dispersion Sci. Technol.* **2015**, 36, 740-746. DOI: 10.1080/01932691.2014.919586.

[38] Sobhaninia, M.; Nasirpour, A.; Shahedi, M.; Golkar, A. Oil-in-water emulsions stabilized by whey protein aggregates: Effect of aggregate size, pH of aggregation and emulsion pH. *J. Dispersion Sci. Technol*. **2017**, 38, 1366-1373. DOI: 10.1080/01932691.2016.1224719.

[39] Seddari, S.; Moulai-Mostefa, N. Formulation and Characterization of Double Emulsions Stabilized by Sodium Caseinate–Xanthan Mixtures. Effect of pH and Biopolymer Concentration. *J. Dispersion Sci. Technol*. **2015**, 36, 51-60. DOI: 10.1080/01932691.2013.873867.

[40] Yadav, N. P.; Srivastava, S.; Sinha, P.; Chanda, D.; Luqman, S.; Tandon, S. Development and evaluation of Aloe vera (L.) Burm. based topical cream formulation. *Ann. Phytomed*. **2014**, 3, 60-65.

[41] Brookfield, CT3 Texture Analyzer Operating Instructions Manual No. M08-372-F1116, Brookfield Engineering Laboratories Ltd., Middleborough, USA, 2011.

[42] Brookfield Ametek, Texture analysis application note: Moisturizing Cream Firmness Test, Brookfield Engineering Laboratories Ltd., Middleborough, USA.

[43] Inoue, Y.; Furuya, K.; Matumoto, M.; Murata, I.; Kimura, M.; Kanamoto, I. A comparison of the physicochemical properties and a sensory test of Acyclovir

creams. *Int. J. Pharmaceut*. **2012**, 436, 265-271. DOI: 10.1016/j.ijpharm.2012.06.023.

[44] Silset, A.; Flåten, G. R.; Helness, H.; Melin, E.; Øye, G.; Sjöblom, J. A multivariate analysis on the influence of indigenous crude oil components on the quality of produced water. Comparison between bench and rig scale experiments. *J. Dispersion Sci. Technol*. **2010**, 31, 392-408. DOI: 10.1080/01932690903110400.

[45] Pan, L. G.; Tomás, M. C.; Añón, M. C. Effect of sunflower lecithins on the stability of water-in-oil and oil-in-water emulsions. *J. Surfactants. Deterg.* **2002**, 5, 135-143. DOI: 10.1007/s11743-002-0213-1.

[46] Codina-Torrella, I.; Guamis, B.; Ferragut, V.; Trujillo, A. J. Potential application of ultra-high pressure homogenization in the physico-chemical stabilization of tiger nuts' milk beverage. *Innov. Food. Sci. Emerg. Technol.* **2017**, 40, 42-51. DOI: 10.1016/j.ifset.2016.06.023.

[47] Assadpour, E.; Maghsoudlou, Y.; Jafari, S. M.; Ghorbani, M.; Aalami, M. Optimization of folic acid nano-emulsification and encapsulation by maltodextrin-whey protein double emulsions. *Int. J. Biol. Macromol*. **2016**, 86, 197-207. DOI: 10.1016/j.ijbiomac.2016.01.064

[48] Hayati, I. N.; Man, Y. B. C.; Tan, C. P.; Aini, I. N. Droplet characterization and stability of soybean oil/palm kernel olein O/W emulsions with the presence of selected polysaccharides. *Food Hydrocolloid*. **2009**, 23, 233-243. DOI: 10.1016/j.foodhyd.2008.01.004

[49] Steffe, J. F. Rheological methods in food process engineering. USA Freeman Press, **1996**, 19-39.

[50] Szeląg, H.; Pauzder, B. Rheological properties of emulsions stabilized by acylglycerol emulsifiers modified with sodium carboxylates. *Colloids. Surf. A. Physicochem. Eng. Asp.* **2003**, 219, 87-95. DOI: 10.1016/S0927-7757(03)00026-8

Table 1 Water contents in fat blends

Fat symbol	Initial water content deermined in enzymatic preparation [%] (wt/wt)	Amount of water added to fat blend in relation to the enzyme mass [%] (wt/wt)	Total water content in fat blend in relation to the enzyme mass [%] (wt/wt)	Total water content in fat blend in relation to the fat blend mass [%] (wt/wt)
F0*		--	--	--
F1		9.5	10.0	0.50
F2		10.5	11.0	0.55
F3		11.5	12.0	0.60
F4	0.5	12.5	13.0	0.65
F5		13.5	14.0	0.70
F6		14.5	15.0	0.75
F7		19.5	20.0	1.00
F8		24.5	25.0	1.25

*F0 represent non-interesterified fat blend

Table 2 Compositions of emulsions based on the prepared fat mixtures

Emulsion	Fat type [%] wt/wt	Emulsifier type	Component [%] wt/wt	
			Emulsifier	Water
E1	F1	DAG and MAG formed during interesterification	Detailed amount provided in the section Results and Discussion (see Table 4)	Up to 100.0
E2	F2			
E3	F3			
E4	F4			
E5	F5			
E6	F6			
E7	F7			
E8	F8			
E9	F0	Lecithin	5.5	
E10	F0	Synthesized DAG	10.0	

Table 3 Fatty acids content in the investigated fats

	14:0	16:0	16:1 (9-cis)	17:0	18:0	18:1 (9-cis)	18:1 (9-trans)	18:2 (all-cis) n-6	18:3 (all-cis) n-6	18:2 (all-cis) n-3	20:0	20:1	Other
HO	N/D	7.8	N/D	N/D	2.7	10.7	0.8	54.8	2.7	18.7	1.2	0.5	0.2
MT	6.4	27.8	1.2	2.0	28.2	23.3	2.7	1.9	N/D	0.6	N/D	N/D	5.8
F0	3.6	19.0	0.7	1.1	15.5	18.0	1.8	27.8	1.7	8.4	0.6	N/D	1.9
F1	2.9	17.2	0.6	1.0	15.6	17.8	1.9	28.6	1.9	9.1	0.7	0.3	2.4
F2	3.2	18.4	0.7	1.2	17.7	18.4	2.1	25.0	1.7	7.9	0.6	0.3	2.7
F3	3.0	17.2	0.7	1.0	15.8	18.3	2.0	27.8	1.8	8.8	0.7	0.3	2.5
F4	3.1	17.6	0.6	1.1	15.7	18.3	2.0	27.7	1.8	8.8	0.7	0.3	2.3
F5	3.3	17.1	0.6	1.0	15.3	18.3	1.8	28.0	1.9	8.9	0.7	0.4	2.6
F6	3.4	17.4	0.7	1.0	15.9	18.6	1.9	27.0	1.8	8.6	0.7	0.4	2.7
F7	3.9	19.0	0.7	1.2	17.2	19.2	1.9	25.0	1.7	7.9	0.6	0.3	1.5
F8	3.6	17.7	0.7	1.0	15.5	18.5	1.7	27.9	1.9	8.7	0.7	0.3	1.8
DAG	4.3	18.1	0.8	1.0	14.0	19.8	1.6	28.0	1.9	8.1	0.6	N/D	1.7
TAG	3.3	17.7	0.7	1.0	15.8	17.8	1.8	27.5	1.9	8.6	0.7	0.4	2.7

Table 4

Part A.
Free fatty acids, mono- , di- and triacylglycerols (FFA, MAG, DAG and TAG) contents and slip melting point of raw fats and interesterified blends

Part B.
The content of produced emulsifiers during the interesterification process present in the final fat mixture - used as an emulsion fat base.

A. Fat characteristics							B. Calculated content of emulsifiers (MAG and DAG) in esterified fat
Fat type		TAG amount [%]	DAG amount [%]	FFA amount [%]	MAG amount [%]	Slip melting point [°C]	Emulsifiers * [%]
Raw fats	HO	96.8±0.7	2.4±0.9	1.0±1.1	N/D	--	--
	MT	95.2±0.5	4.6±0.8	0.3±0.9	N/D	--	--
NIE blend	F0	95.6±0.9	3.4±0.6	0.6±0.7	N/D	47.1±0.1	--
EIE blends	F1	74.4±0.4	15.6±0.3	8.6±0.8	1.4±1.0	38.5±0.1	5.1
	F2	73.6±0.7	15.8±0.9	9.2±1.0	1.4±1.3	37.8±0.3	5.2
	F3	73.9±0.8	16.2±0.8	8.8±0.9	1.1±0.8	35.0±0.2	5.2
	F4	73.1±0.7	17.1±0.5	9.1±1.1	0.7±0.9	35.4±0.7	5.3
	F5	70.1±0.6	19.1±0.7	9.9±0.8	0.9±1.2	35.6±0.1	6.0
	F6	68.4±0.7	19.1±0.8	11.5±0.9	1.0±0.8	32.9±1.0	6.0
	F7	64.3±0.9	20.0±0.8	13.6±0.7	2.1±0.9	23.5±0.3	6.6
	F8	63.1±0.8	22.1±0.5	14.2±0.6	0.6±0.7	22.2±0.5	6.8

HO – Hemp Oil; MT – Mutton Tallow; N/D – not detected . Emulsifiers content in emulsion on a basis on their content in fat blends*

Table 5 Particle size of emulsions

Emulsions shelf-life [weeks]	**E1**	**E2**	**E3**	**E4**	**E5**	**E6**	**E7**	**E8**	**E9**	**E10**
0	5.8	4.6	4.2	6.1	4.3	4.4	3.4	4.0	4.1	7.5
1	5.8	4.7	4.3	6.2	4.3	4.8	3.5	4.1	4.2	10.2
2	5.8	4.7	4.3	7.1	4.7	4.8	3.5	4.1	4.8	10.9
3	6.5	5.0	4.5	7.7	4.7	4.9	3.5	4.2	5.7	13.7
4	7.6	6.5	4.9	7.8	5.3	5.1	3.7	4.5	6.1	14.8

Figure 1 Polarized light microscopy images of fat blends before and after enzymatic interesterification

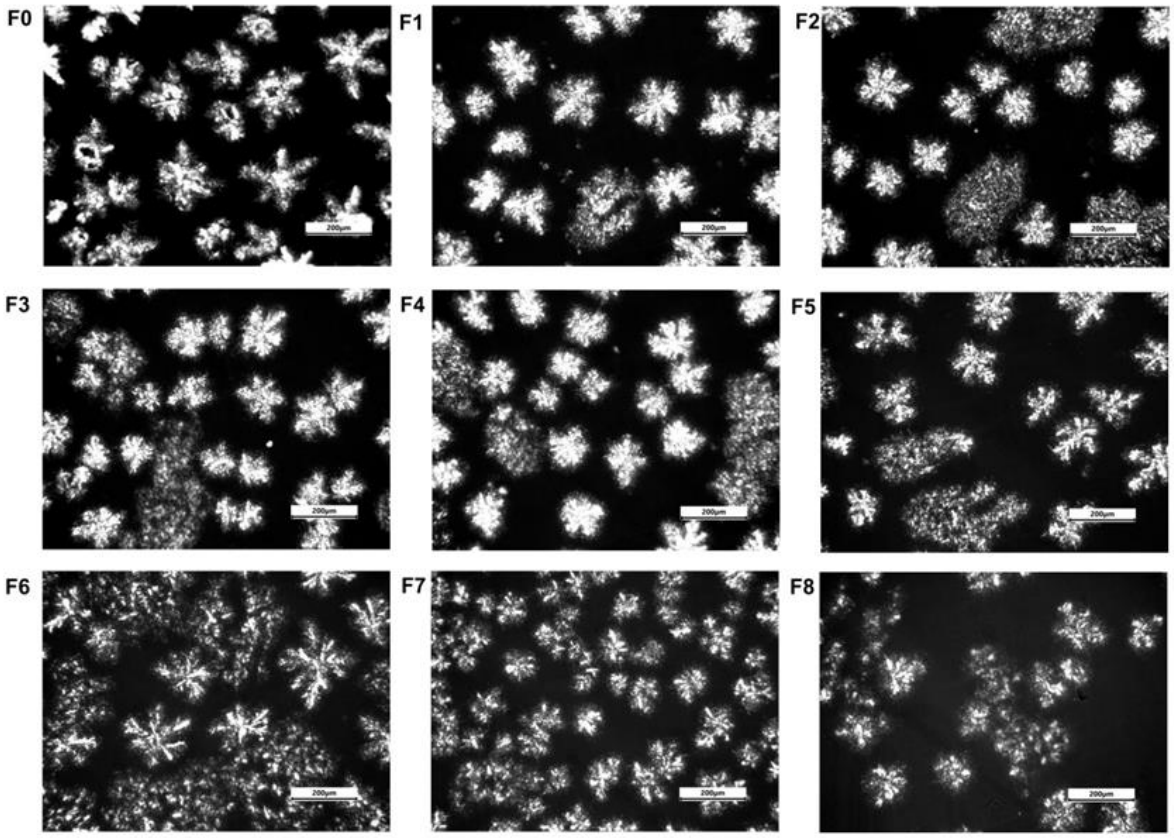

Figure 2 pH values of the prepared emulsions (as a mean value of 3 determinations ± SD)

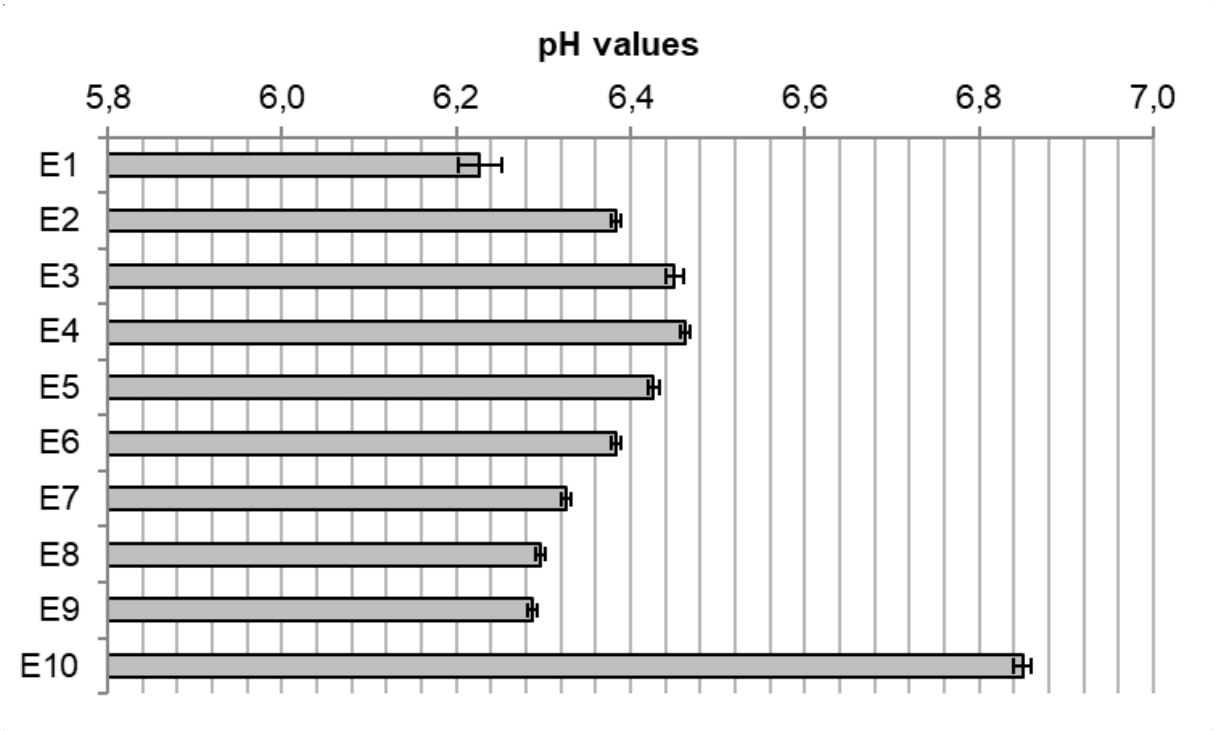

Figure 3 Texture parameters of the freshly prepared and stored emulsions (30 days, 5°C)

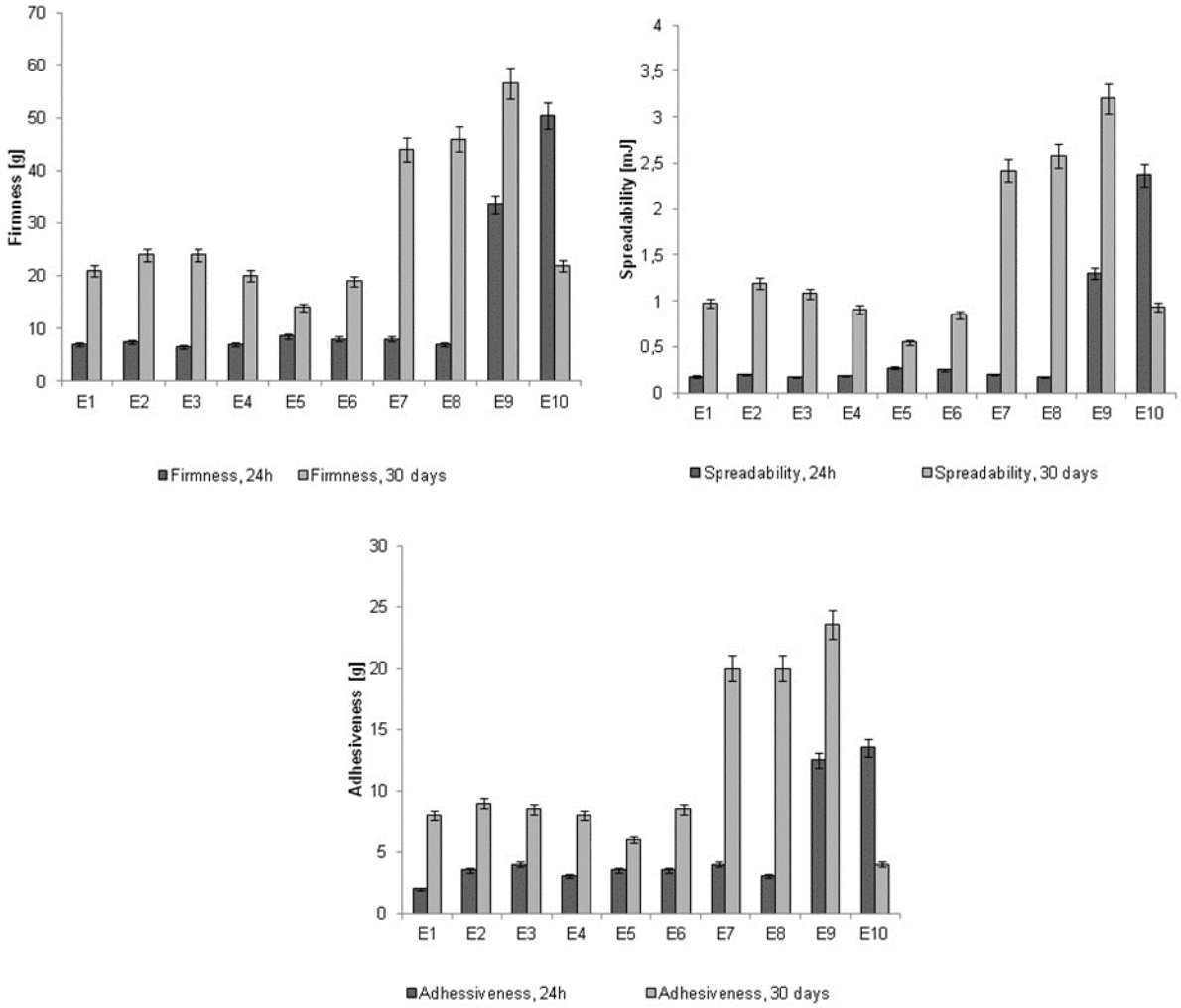

Figure 4 Delta backscattering values [%] for emulsions E1 – E10

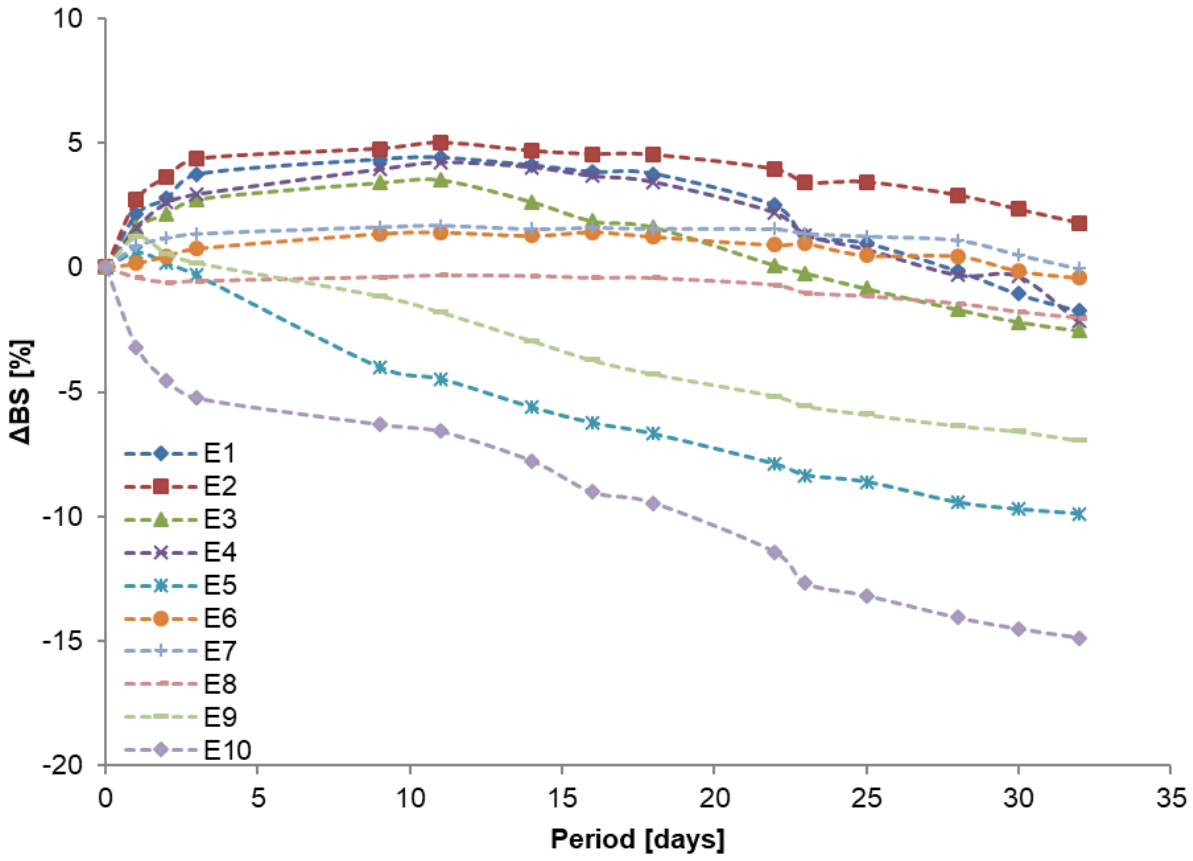

Figure 5 Kinetics of destabilization of emulsions E1 – E10

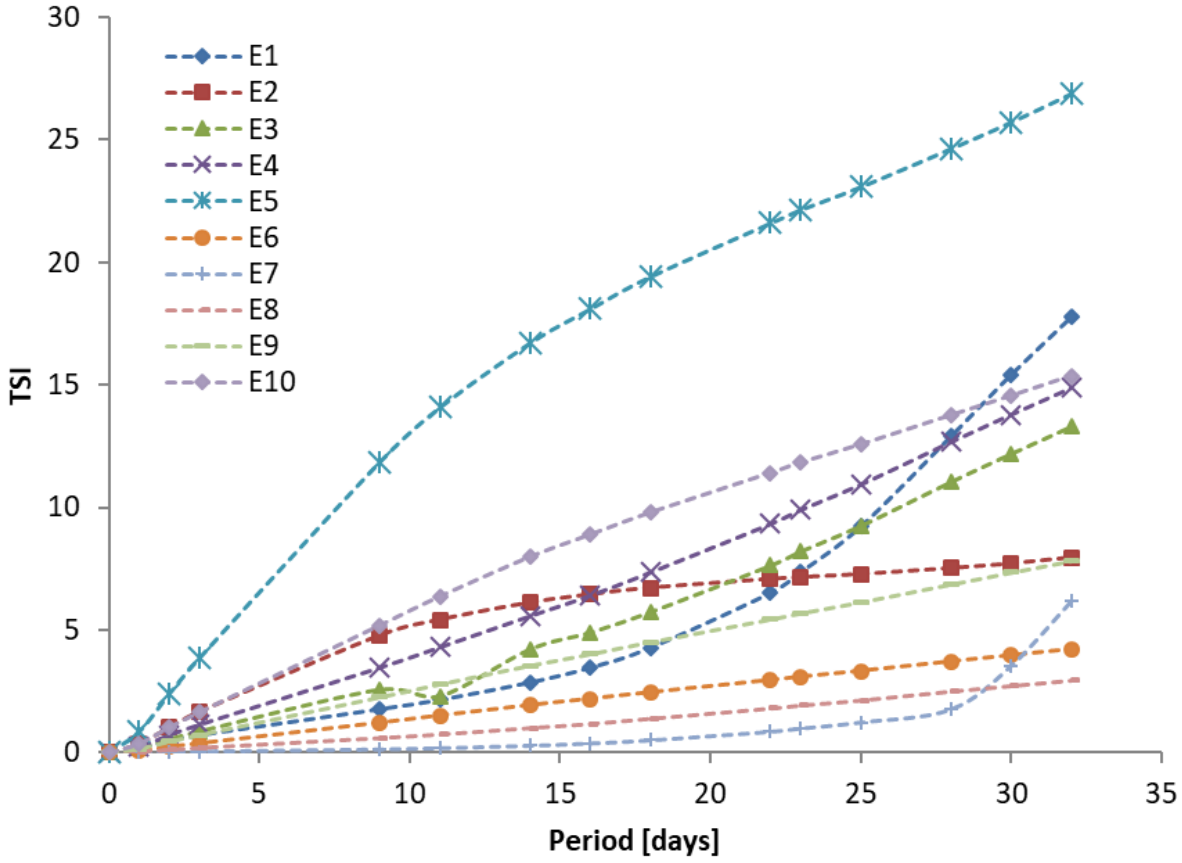

Figure 6 Viscosity of the prepared emulsions after 24h and 1 month from their preparation (as a mean value of 3 determinations ± SD)

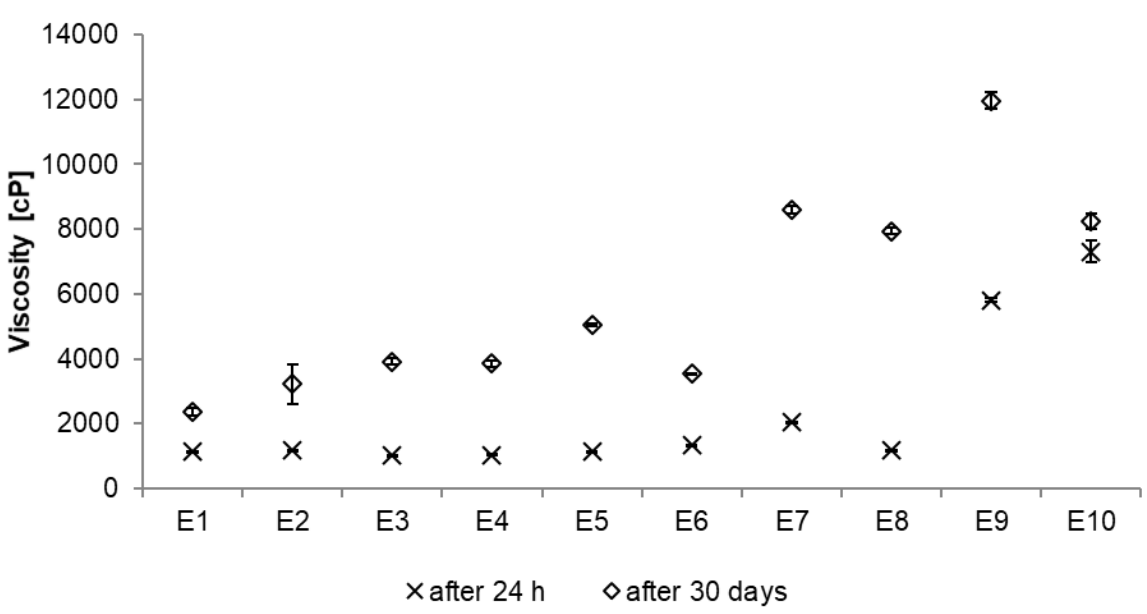

Figure 7 Dependencies of storage (G') and loss (G'') moduli on the applied strain of the investigated emulsions. Measurements were performed at constant angular frequency of 1rad/s.

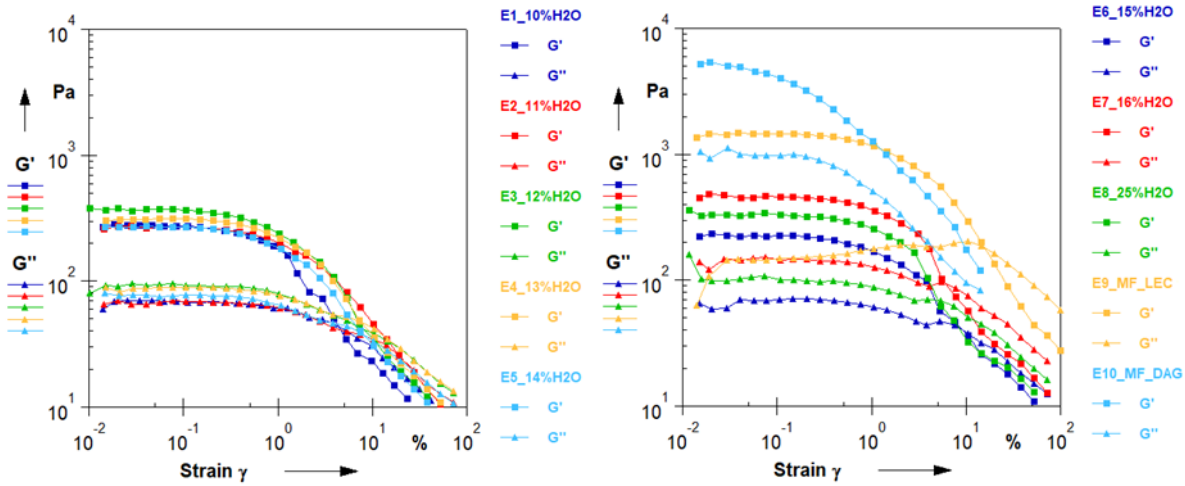

Figure 8 Dependencies of storage (G') and loss (G'') moduli on angular frequency (at constant strain amplitude 0.1%)

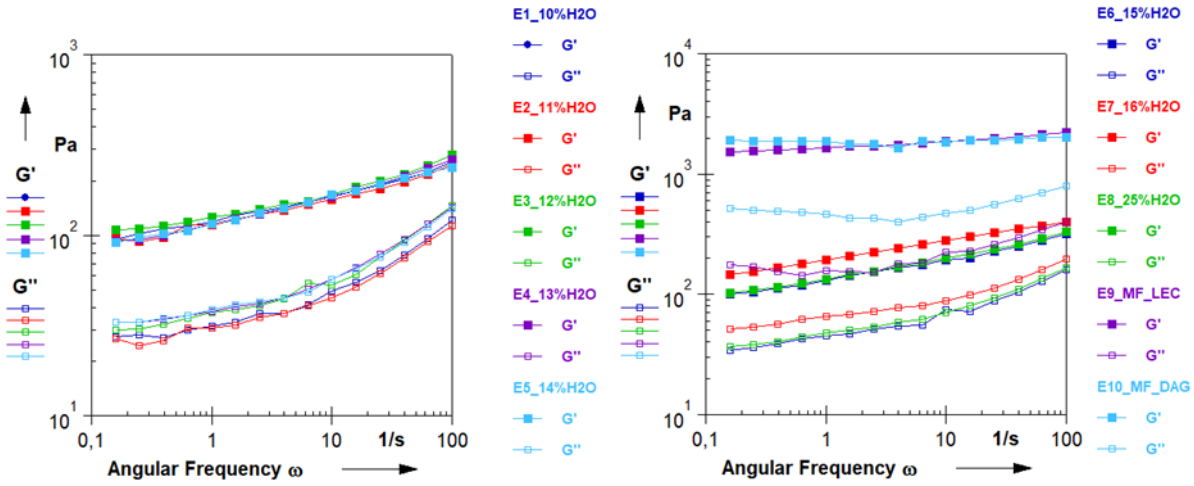

Figure 9 Dependence of damping factor (tanδ) on angular frequency, measured for the investigated emulsions.