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ASSESSMENT OF A STABLE COSMETIC PREPARATION BASED ON ENZYMATIC INTERESTERIFIED FAT, PROPOSED IN THE PREVENTION OF ATOPIC DERMATITIS

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Abstract: Atopic dermatitis is one of the most common skin disorders seen in infants, children and adults. Proper prevention might slow the atopic symptoms. The purpose of the work was a sensory analysis, an evaluation of moistening properties and stability of emulsions based on an enzymatic interesterified fat blend (mutton tallow and walnut oil) and homogenized at different revolutions and different contents of thickener. The emulsions were evaluated with respect to sensory and skin moisturizing properties by 78 respondents. Stability tests, particle size, distribution, dispersity index, morphology structure of the emulsions were determinated too. Taking into consideration all properties of the emulsions, emulsion IV (containing 0.9 g carboxymethyl cellulose and homogenized at 18000 rpm) and emulsion V (1.5 g of carboxymethyl cellulose and homogenized at 24000 rpm) were found to be of optimum composition. The emulsions exhibited good stability, were highly rated in sensory terms and displayed optimum moistening properties. It has been proven that model emulsions based on interesterified fats containing partial acylglicerols, with optimum carboxymethyl cellulose content and specific revolutions at the time of homogenization are an opportunity for developing preparations targeted at skins requiring special care (e.g., with atopic dermatitis or psoriasis). The work proved the use of enzymatic process to create the emulsifier, which represents the innovative contribution of this work. Also it showed an additional application of enzymatic interesterified fats which since has been used only in food industries.

Keywords: interesterification, moisture, sensory evaluation, stability of emulsions, carboxymethyl cellulose, mutton tallow, walnut oil

Fats have a significant impact on the proper functioning of the skin. This is mainly due to the role of the building blocks of these ingredients. The stratum corneum is the main component of the protective skin for maintaining the balance between external and internal environment of the body. This is due to its characteristic structure in which keratinocytes are arranged along the lines of a brick wall. These considered dead cells (without nuclei) are connected by interstitial binder formed from a mixture of polyunsaturated fatty acids, cholesterol and ceramides (sphingolipids). Keratin, fats and anatomical structure effect on the skin barrier. It provides better penetration of exogenous substances through the skin and slow down the diffusion of water from the deeper layers of the dermis, thereby regulating the degree of hydration of the skin (1).

However, the natural fats are not always best suited for people's demands. Lipid scientists and technologists have been engaged in improving the quality of fats using different kinds of fat modification. One of them can be enzymatic interesterification where lipases are used as the catalysts. Generally, lipases exhibit wide substrate specificity, stereoselectivity and enantioselectivity and therefore, are industrially significant (2). In enzymatic interesterification, using regiospecific (1,3 SN- or 2 SN-specific position) and fatty acid specific lipases as catalysts, the positioning of acyl groups on the glycerol backbone of triacylglycerols is controlled and a desired acyl group can be guided to a specific position (3).

Water content is a very important factor in environments where lipase as a catalyst is intro-

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duced. Enzymatic interesterification reaction systems are obliged to contain a certain amount of water, because the reaction is performed on the water/oil boundary phase (4). In this case water has two functions: it is required by the enzyme to maintain an active hydrated state, and it is a reactant for producing monoacylglycerols (MAGs), diacylglycerols (DAGs) and free fatty acids (FFAs) from triacylglycerols (TAGs) by hydrolysis; however, for better synthesis, the water content should be kept as low as possible (5). Therefore, to obtain a larger polar fraction (MAGs and DAGs) it is necessary to add more water to the enzymatic preparation during interesterification. MAGs and DAGs can play a key role in governing the partial coalescence rate and, hence, probably in improving the structure of the emulsions (6).

Emulsion systems are metastable colloids made out of two immiscible fluids, with one of the liquids dispersed as small spherical droplets (7). Emulsions (oil-in-water (o/w) and water-in-oil (w/o)) are widely used in cosmetic and pharmaceutical preparations due to their excellent solubilizing capacities for lipophilic and hydrophilic active ingredients and application acceptability (8). According to Ramanauskiene et al. (9), the base of the preparation should be aqueous, since water is absorbed into the skin, the skin swells and softens, which increases the permeability of the skin. Humidification of the stratum corneum is the most important factor increasing the penetration of the active substance in the target site.

Those systems are typically stabilized by several types of surface active components. Taking into account the preferences of consumers and skin problems resulting from the diversity of substances added to cosmetics, it becomes reasonable with possible limitation of these components or the introduction of totally natural substances which are not regarded as potential allergens. According to Nazir et al. (10), the safety profile of personal care have to be considered, and the excessive usage of surfactants and/or thickeners can lead to safety concern.

One of the most promising strategies to achieve the stable emulsions is adding emulsifiers (MAGs and DAGs formed during interestrifiation process) to the emulsion. The use of mono- and diacylglycerols as non-ionic emulsifiers in the food, cosmetic and pharmaceutical industries and as synthetic chemical intermediates has been a growing research area in recent years (11).

Furthermore, they have a status generally recognized as safe, which contributes to their larger application. Also, the antimicrobial activities of particular types of monoglycerols such as monolaurin, monomyristin, monolinolein and monolinolenin have been reported (12). Since, MAGs and DAGs were obtained by the glycerolysis of triacylglycerols or by the direct esterification of glycerol with fatty acids. Both reactions always lead to products, which are mixtures of mono, di and triacylglycerols and some unreacted substrates (13). It should be noted that synthesis of mono- and diacylglycerols using an enzymatic process is an environmentally friendly approach (14). Using enzymatic interesterification, hard fat like mutton tallow (less popular fat used in a direct application to cosmetics and rather considered as a by-product) can be enriched in monoenoic or polyenic fatty acids from vegetable oils (15) such as walnut oil. However, as opposed to the majority of animal fats, mutton tallow has a much more beneficial profile of fats. Generally, it contains more unsaturated fatty acids, the presence of saturated palmitic acid is two or even three times less than in another hard fat as beef tallow. The important role has the presence of conjugated linoleic acids (CLAs) in mutton fat. CLAs have been associated with important health benefits such as anti-carcinogenicity and alteration of body composition. Besides, CLAs have a positive effect on the immune system and help to prevent the development of atherosclerosis, diabetes and osteoporosis (14). The combination of above mentioned fats by using the enzymatic modification gives the possibility of achieving a new structured fats with a beneficial fatty acid composition and with the presence of MAGs and DAGs.

The quantity of water in an enzymatic preparation to be added in the interesterification process in order to generate a quantity of emulsifiers sufficient to stabilize an emulsion system was determined at approximately 13%. This hypothesis was based on earlier review of the literature and previously conducted studies (14).

The current paper is aimed at a sensory analysis, an evaluation of moistening properties and stability of emulsions based on an interesterified fat blend and homogenized at different revolutions and different contents of viscosity modifier. Both the parameters significantly affect quality of emulsion and, above all, average size of dispersion system particles, which in turn translates into adequate penetration and produces appropriate moistening of the skin.

EXPERIMENTAL

Materials

Walnut oil (WO) was purchased at the market (producer OLEOFARM, Poland). The composition

of walnut oil's main fatty acids was as follows: C16:0 (7.1%), C16:1 (0.3%), C 18:0 (2.6%), C 18:1 cis 9 (20.0%), C 18:2 n-6 (56.0%), C 18:3 n-3 (12.6%). Also, it contains vitamins such as A, E, B and tocoferols, which make it more attractive than others animal fats (16).

Mutton tallow (L) was obtained from the company Meat-Farm, Stefanowo, Wólka Kosowska, Poland. It was laboratory refined, bleached and deodorized under vacuum at 105°C. Its composition of main fatty acids was: C 14:0 (1.7%), C 16:0 (18.2%), C 16:1 cis 9 (0.9%), C17:0 (1.7%), C 18:0 (30.2%), C 18:1 cis 9 (31.9%), C 18:2 all cis (1.8%), C18:2 cis-9, trans-11 (0.7%), C 18:3 all cis (0.7%), C 20:0 (0.2%), C 20:1 cis 11 (0.1%), total n-3 (0.5%), total n-6 (1.7%).

Lipozyme RM IM (Novozymes, Bagsvaerd, Denmark) was used as a catalyst during enzymatic interesterification. The detailed description of this preparation is given in the reference (14).

Carboxymethyl cellulose was used as a thickener for each emulsion solutions (producer Mikro-Technik GmbH & Co. KG, Germany). *Aloe vera* (producer FLP, Scottsdale, Arizona, USA). Citric acid (producer Jungbunzlauer, Basel, Switzerland). Sodium benzoate (producer Orff Food Eastern Europe, Marki/Warszawa, Poland).

Methods

Enzymatic interesterification of fats

Mutton tallow was mixed at 70°C under nitrogen with walnut oil (L : WO) in the proportions 3 : 3 (w/w). Flask containing the fat blend was placed in a thermostated mineral oil shaker bath (type 357, producer Elpin plus, Lubawa, Poland). After thermal equilibration of the fat blend at the desired temperature of 60°C, 8 wt-% of Lipozyme RM IM was added to the blend. To obtain an established quantity (of polar fraction responsible for good stability of emulsions) in the reaction environment *via* hydrolysis, 13% of water was added to the enzymatic preparation. That quantity of polar fraction as: MAGs and DAGs was desired because those fats with a higher quantity of the partial glycerols are used in emulsion systems as emulsifiers. The interesterification was performed with continuous shaking for 6 h. After a predetermined time of interesterification, the samples were filtered to stop the reaction.

The composition of fatty acids (FA) of walnut oil and mutton tallow was determined using gas chromatography (GC) after conversion of the fats to fatty acid methyl esters (FAMEs). The detailed procedures of that determination are included in the reference (14).

Polar fraction content was determined using a column chromatography on silica gel, SG 60, 70-230 mesh, Merck, Darmstadt, Germany, according to the ISO standard (17). The content of polar fraction is placed in Table 1.

Emulsion preparation and analysis

Emulsions were synthesized according to the recipes prepared on the basis of our own experience

Table 1. Composition and parameters of emulsions

Component [%]	**Type of emulsion**					
	E I	E II	E III	E IV	E V	EVI
Carboxymethyl cellulose	0.3	1.5	0.6	0.9	1.5	0.3
Content of polar fraction (MAGs, DAGs, FFAs)	28.0 ± 1.08					
Fat blend	30.0					
Sodium benzoate	0.3					
Aloe vera	0.2					
Citric acid (to pH 5.5)	q.s.*					
Water	69.2	68.0	68.9	68.6	68.0	69.2
Parameter						
Mixing speed, [rpm]	6000	6000	12000	18000	24000	24000
Mixing time, min	4					

Legend: E I - VI -emulsions (I-VI); *q.s. - quantum satis (as much as is enough)

and optimized with the software based on Kleeman's method (14, 18) (Table 1). Appropriate amount of carboxymethyl cellulose was dispersed in distilled water. Both solutions (oil and aqueous phases) were heated at 50-55°C in a water bath. Homogenization of the aqueous phase with the oil phase was achieved with a high shear mixer at the appropriate mixing speeds (given in Table 1) for 4 min. Finally, the emulsions were cooled to room temperature and a stabilizer (sodium benzoate) was added. The volume of each mixtures was 100 g.

Determination of skin humidification

Skin humidification was measured by means of a CM 825 Corneometer (Courage + Khazaka Electronic GmbH, Köln). Respondents gave their written consent for taking measurements of functional parameters of the skin before starting the study. Testing of each emulsion was carried out on two groups of women: 42 women aged 18-35 and 36 women of 40-50. Because presented preparations were model emulsions, therefore women with no special symptoms of atopic skin but having dry and sensitive skin were invited to the test. The reading of skin humidification was taken five times to get valid calculation. More than half of respondents are the women educated on the faculty of Health Sciences and Physical Culture at the University of Technology and Humanities in Radom, Poland. The remaining women represented the profession of: pharmacy technician, dental hygienist, dental assistant, medical rescuer (students of medical schools). Research was conducted under the control of a trained person. Research for all volunteers lasted for 3 weeks (01 December 2014 – 21 December 2014). Emulsions were stored during the whole period of the measurement in the refrigerator (3-5°C).

Procedure of skin humidification determination

Testing began on clean, degreased skin, which constituted the point of reference and zero time of the effect on the skin. 0.01 g of the emulsion was applied to the same 20 × 20 mm fragment of the forearm skin and left there for 5 min. The probe of the cornea before and after the measurement was always wiped with a clean cotton cosmetic swab. The region of the skin before and after each measurement was wiped with a swab too. Humidification was examined at 22 ± 1°C after 5 min and at intervals of 15 min until 90 min after the moment the skin was cleaned of the emulsion. Variations of skin humidification were calculated by the following formula, averaging results for all subjects involved in the test:

$$ZN_t = \frac{N_t - N_{PKt}}{N_{PKt}}$$

where: ZN_t is a change of skin humidification over time "t"; N_t is average skin humidification after "t" for a place with the tested preparation on; N_{PKt} is average skin humidification after "t" for the control point.

Sensory determination

Test continued for 2 weeks in the period from 01 to 14 December 2014 and was conducted three times a week. Six preparations were subjected to the sensory analysis The emulsions were assessed for the following characteristics: consistency homogeneity, cushion effect, distribution, smoothing, viscosity, greasiness and absorption. The assessment was based on a five-point scale, as follows: 1 – quality of evil, 2 – insufficient quality, 3 – satisfactory quality, 4 – good quality, 5 – very good quality. The detailed procedure and the scoring scale according to above mentioned analysis is given in Table 2. Sensory analyses were conducted in the presence of the person responsible for sensory assessment (proper behavior during measurements). The survey was supplemented with a question concerning the most suitable emulsion in the respondent's opinion.

Determination of emulsion stability using the centrifugal test

Determination was measured in the centrifugal machine at 3000 rpm. Test tubes were filled with 10-15 mL of the emulsion and then centrifuged for 30 min, with the state of emulsion checked every 10 min. If the emulsion remained homogeneous after 30 min it was considered to have proper stability.

Determination of emulsion stability using the temperature test at 35°C and 3°C

All preparations were stored in the dryer at 35°C (± 0.3°C) and in the refrigerator at 3-5°C alternately for five days, with a change every 24 h. If the emulsion remained homogeneous in such conditions, it was considered to have proper stability.

Determination of average particle size and particle size distribution of fat emulsions

The average droplet size and distribution was determined after 48 h from the manufacturing. For measurements the emulsions were diluted 1 : 200 with distilled water. Droplets size was measured in the range 0.12–704 μm by laser scattering using a Microtrac Particle Size Analyzer (Leeds & Northrup, Philadelphia, USA), total time of determination of each sample was 30 s.

Table 2. Selected quality parameters and guidelines for sensory analysis of the cosmetic emulsions tested.

Parameter	Definition	Test	Points (1-5)
Consistency	Determines the density, viscosity and stability of emulsion.	Dip your finger in a emulsion container to a depth of approximately 0.5 cm^3 and pull it out quickly	1 - impossible to apply 2 - too thick to apply to the hand 3 - cosmetic is hard to apply 4 - easy to apply yet flowing can be observed 5 - cosmetic is easy to apply, not flowing
Homogeneity	Specifies the homogeneity and smoothness of the emulsion's surface, determines the quality of emulsion.	Assess the homogeneity and smoothness of emulsion, and then apply a small amount onto the skin and spread it in order to check it for lumps or air bubbles.	1 - formulation components are not dissolved. 2 - heterogeneous 3 - observable and palpable clots and air bubbles in the substance and on the skin when applied 4 - homogeneous, no clots and few air bubbles, forms an uneven layer 5 - completely homogeneous, no clots or air bubbles, forms a smooth layer on the skin when applied
Cushion effect	Specifies the amount of emulsion felt between fingers while rubbing, the more the product is felt, the stronger the cushion effect is.	Put approximately 0.5 cm^3 of emulsion between your fingers, rub it and identify the felt amount.	1 - highly perceptible substance 2 - more perceptible substance 3 - somewhat perceptible substance 4 - weakly perceptible substance 5 - imperceptible substance
Distribution	Specifies whether it is easy to spread emulsion on the skin.	Put approximately 0.5 cm^3 of emulsion on the inner side of the forearm and evaluate the resistance of the emulsion when distributed on the skin.	1 - impossible to spread 2 - difficult to spread 3 - incomplete cover, good spreading 4 - little resistance to spreading 5 - no resistance to spreading
Smoothing	Specifies the level of the skin smoothness after emulsion application.	Apply a small amount of emulsion on the inner side of the forearm, rub it in, and after 15 min, assess the smoothness of the skin by comparing the parts where the emulsion has been applied with the other parts.	1 - very rough skin 2 - rough skin 3 - the skin surface is as smooth as that of the reference standard 4 - smoother and softer skin surface than of the reference standard 5 - very smooth and soft skin surface
Viscosity	Determines the degree of the viscous layer left on the skin after application.	Apply a small amount of emulsion on the inner side of the forearm, rub it in, put a finger of the opposite hand and evaluate the viscosity.	1 - high skin viscosity 2 - increased skin viscosity 3 - palpable skin viscosity 4 - low skin viscosity 5 - no palpable skin viscosity
Greasiness	Specifies the amount of a greasy film left by emulsion when applied onto the skin.	Apply a small amount of emulsion on the inner side of the forearm, rub it in and determine whether the emulsion leaves a greasy film.	1 - a compact, greasy film after application 2 - greasy film on the skin directly on application 3 - thin, greasy film on the skin after application 4 - weak sense of greasiness, no film on the skin 5 - no sense of grease or film formation on the skin after application
Absorption	Specifies the rate of emulsion absorption	Apply a small amount of emulsion on cleaned skin and assess the time of its absorption	1 - very poor absorption for more than 5 min. 2 - poor absorption from 3 min to 5 min 3 - average absorption from 1 min to 3 min. 4 - good absorption from 30 s to 1 min. 5 - very good absorption below 30 s.

Determination of dispersion index

The dispersion index (K) was calculated according to the following formula:

$$K = \frac{D_{90} - D_{10}}{D_{50}}$$

where: D_{90}, D_{50} and D_{10} are droplet diameters (mm) read from the differential volume (90%, 50%, 10% of particles that have diameters lower than the stated value, respectively).

Determination of type of emulsion (digestion test)

To a beaker containing 100 g of distilled water it was added 1 g of the emulsion and placed on a magnetic stirrer. The stirrer was turned on and adjusted to the rotational speed of 100 rpm. The time after the droplet of the emulsion was dissolved was noted or noted that such process did not take place. Determination was carried out at room temperature. As the final result, it was considered the average time of two independent measurements for each emulsions.

Morphology of emulsions

Distribution of emulsified oil droplets were observed in the emulsion solutions in the fifth day after preparation using a usual optical microscope (BIOLAR EPI BE 2403 PZO, Warszawa, Poland, lens Nikon 5/0.12; 20/0.4 – Poland, camera ToupCam SCMOS 03000KPA 3.0 Mp 1/2, 7 222, software ToupView 3.7 Tungsten sets of Koehler's rule). The samples were tested with 5× objective and magnification 62.5× for EI, EII, the remained emulsions with 20× objective and magnification 250×

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Statistical analysis

The results were calculated by one-way ANOVA. The Duncan test was used to assess the differences between means. The level of $p < 0.05$ was considered significant. Statgraphics plus 4.0 package (Statistical Graphics Corp., Warrenton, VA, USA) was used.

Table 3. Sensory profile of emulsions for the respondents aged 18-35 years.

Feature	Type of emulsions					
	E1	E2	E3	E4	E5	E6
Consistency	4.7ab ± 0.47	4.4a ± 0.66	4.9b ± 0.33	4.8b ± 0.39	4.7ab ± 0.49	4.5a ± 0.51
Homogeneity	4.7b ± 0.51	4.0a ± 0.57	4.8b ± 0.38	4.8b ± 0.43	4.8b ± 0.41	4.8b ± 0.41
Cushion effect	4.5ab ± 0.51	3.3a ± 0.85	4.8b ± 0.43	4.8b ± 0.41	4.8b ± 0.41	4.6ab ± 0.55
Distribution	4.9b ± 0.28	4.2a ± 0.86	5.0b ± 0.00	4.9b ± 0.28	4.9b ± 0.36	4.8b ± 0.38
Smoothing	4.6a ± 0.49	4.6a ± 0.49	4.7a ± 0.44	4.8a ± 0.38	4.7a ± 0.47	4.8a ± 0.43
Viscosity	4.5bc ± 0.70	3.7a ± 0.56	4.9d ± 0.32	4.3b ± 0.70	4.7cd ± 0.59	4.8cd ± 0.49
Greasiness	4.1b ± 0.73	3.3a ± 0.91	4.8d ± 0.47	4.6cd ± 0.65	4.3bc ± 0.76	4.6cd ± 0.55
Absorption	3.7b ± 0.92	3.2a ± 0.81	4.9c ± 0.36	4.6c ± 0.49	4.5c ± 0.66	4.6c ± 0.56

a, b, c... different letter in columns indicate the mean values that differ statistically significantly ($p < 0.05$)

Table 4. Sensory profile of emulsions for the respondents aged 40-50 years.

Feature	Type of emulsions					
	E1	E2	E3	E4	E5	E6
Consistency	4.7ab ± 0.46	4.7ab ± 0.45	5.0c ± 0.00	4.9bc ± 0.33	4.8bc ± 0.39	4.6a ± 0.50
Homogeneity	4.9b ± 0.24	4.1a ± 0.73	5.0bc ± 0.00	4.9b ± 0.28	4.9b ± 0.24	4.8b ± 0.43
Cushion effect	4.7b ± 0.46	3.2a ± 0.72	5.0c ± 0.00	5.0c ± 0.00	5.0c ± 0.00	4.6b ± 0.49
Distribution	5.0c ± 0.00	3.1a ± 0.76	5.0c ± 0.00	5.0c ± 0.00	4.7b ± 0.44	5.0c ± 0.00
Smoothing	4.5a ± 0.51	4.8b ± 0.38	4.9b ± 0.28	4.9b ± 0.32	4.9b ± 0.28	4.9b ± 0.32
Viscosity	4.7c ± 0.47	3.9a ± 0.28	4.9d ± 0.24	4.2b ± 0.51	4.7c ± 0.57	4.8cd ± 0.45
Greasiness	4.1b ± 0.81	3.4a ± 0.91	4.9c ± 0.36	4.7c ± 0.53	4.5c ± 0.82	4.6c ± 0.73
Absorption	3.7b ± 0.76	2.9a ± 0.84	4.9d ± 0.32	4.5c ± 0.66	4.5c ± 0.74	4.7cd ± 0.54

a, b, c... different letter in columns indicate the mean values that differ statistically significantly ($p < 0.05$)

RESULTS AND DISCUSSION

Natural fats, with triacylglycerols as chief components, display greater affinity to skin than e.g., mineral oils or silicones. This is a result of the structure and function of these substances, similar to those composing the skin. Natural fats externally applied to the skin exhibit dermatological activity which protects the body from water losses and regenerate damaged lipid epidermal barrier, rejuvenating the skin's appearance. Added to all this, the lipid phase is a carrier of active substances that may penetrate deeper skin layers and influence their condition (19). Adding fats, sources of oleic, linolic, linoleic acids or CLA, to the emulsion helps to produce innovative systems enriched with those ingredients, important to the skin. It is particularly important if the atopic skin is considered. According to authors (20) atopic dermatitis is a common chronic skin disorder which is characterized by increased trans epidermal water loss so it needs constant special care.

Digestion testing of the emulsion in water showed that all the emulsions produced were o/w systems. Digestion time of emulsions I, III, IV, VI was below 15-20 s, reaching 35 s for emulsion V and the longest duration of 47 s for emulsion II.

Sensory analysis in both the age groups (18-35 and 40-50) gave comparable results (Tables 3, 4). Emulsions E III, IV, V were highly rated in respect of all the characteristics under analysis (consistency, homogeneity, cushion effect, distribution, smoothing, viscosity, greasiness and absorption). Their consistency was homogeneous, smooth, without clotting or air bubbles, helping to spread the preparation uniformly across the skin. This was confirmed by statistical analysis as well, showing these emulsions to have best consistencies, statistically

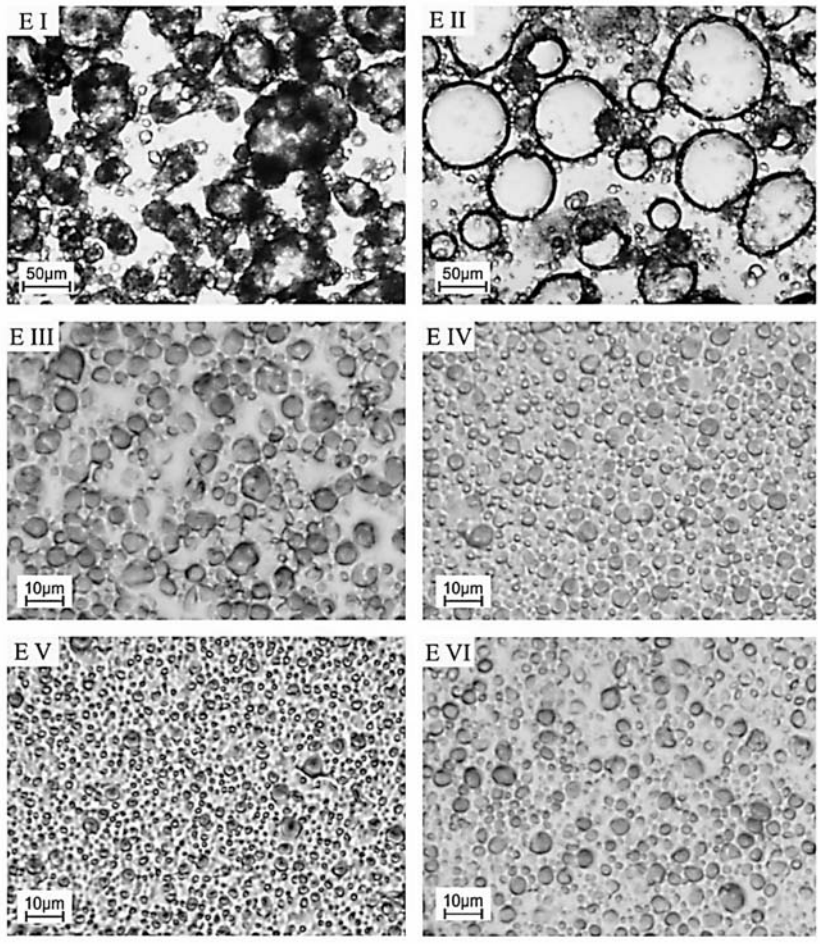

Figure 1. Optical microscopic image of emulsions E I – E VI in the fifth day from manufacturing

significantly better than emulsions I, II, VI. All the women decided that emulsion VI had the poorest consistency.

Regardless of the age group, the evaluators also said emulsions E IV and E V were easy to apply and did not flow off the skin. This was probably due to maximum quantities of viscosity modifier additions (E IV – 0.9%; E V - 1.5%) (Table 1). The high quality of these emulsions was confirmed by the microscope image analysis which showed the greatest homogeneity of these systems (Fig. 1). Some evaluators pointed to low stickiness and a low sense of skin fattiness immediately on application. These emulsions were absorbed between 20 s and 1 min. Correct composition and homogenization of emulsions IV and V were confirmed by their minimum average particle size and the lowest dispersion coefficient (3.166 μm; 1.3 for emulsion IV; 1.3 and

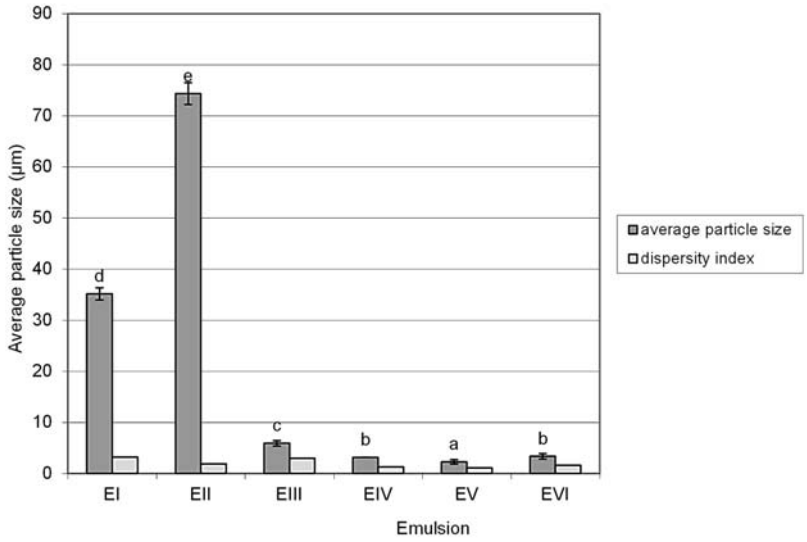

Figure 2. Average particle size (mean ± SD) and the value of dispersity index of emulsions: E I – E VI 48 h from manufacturing a,b,c.. – different letters indicate mean values that differ statistically significantly ($p < 0.05$)

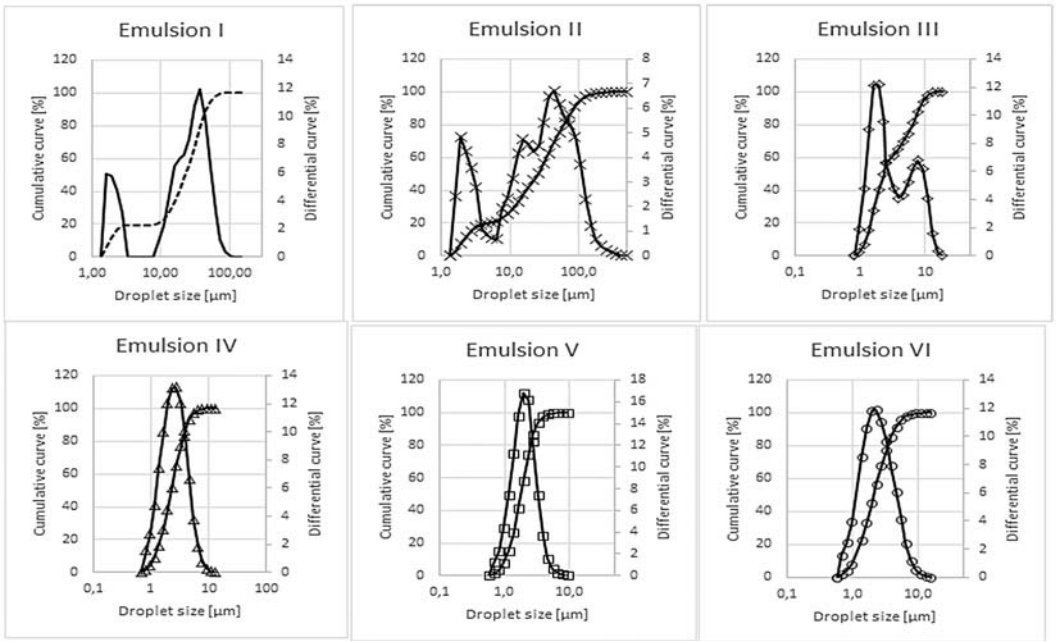

Figure 3. Percentage of particles of given sizes and accumulated distribution in the emulsion: E I to E VI 48 h from manufacturing

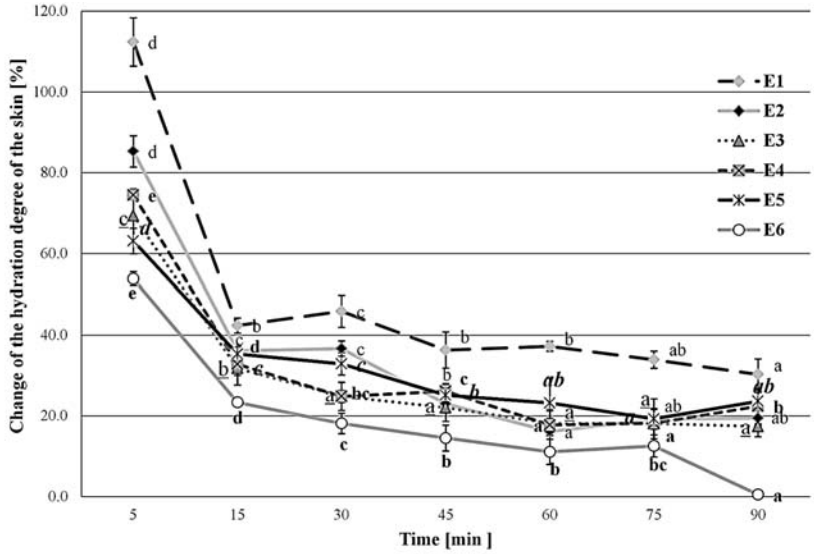

Figure 4. Changes of the hydration degree of the skin with the passage of time for the women aged 18-35 years (mean ± SD). a,b,c. – as given in Figure 2

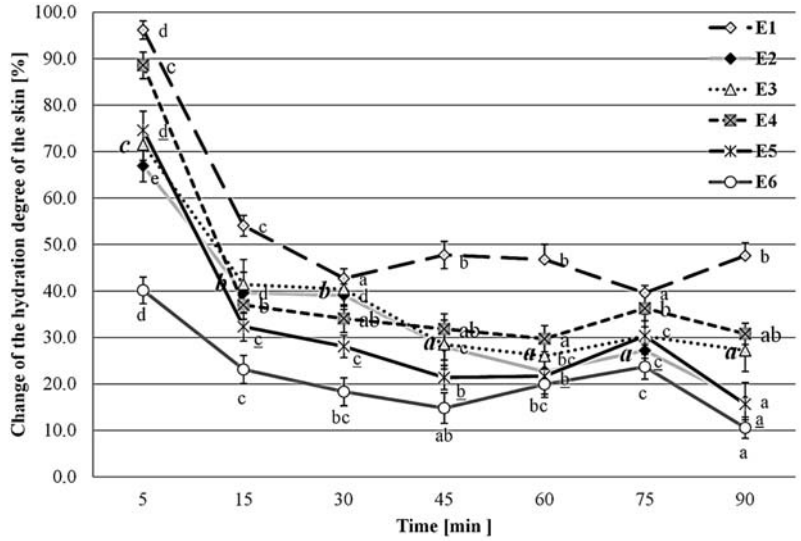

Figure 5. Changes of the hydration degree of the skin with the passage of time for the women aged 40-50 years (mean ± SD). a,b,c.. – as given in Figure 2

2.277 µm; 1.1 for emulsion V, respectively) (Fig. 2). Their distributions were of a monodisperse nature. Analysis of cumulative distribution of oil droplet showed that diameters of 90% of emulsion IV oil phase balls were below 4 µm, while 90% of those of emulsion V were below 3 µm (Fig. 3). Generation of such small particles suggests that dispersed phase substances are capable of penetrating deeper skin layers, not remaining merely in the superficial layer (21). Unsaturated fatty acids, linolic and linoleic, added to the fat mix at the time of interesterification can be transported towards deeper skin levels on application of the emulsion to the skin. Administration of such preparations may prevent a number of skin imperfections and support proper skin function. These acids are universally known to be crucial diet components and their deficits may result in such skin conditions as psoriasis or atopic dermatitis and excessive skin dehydration (22, 23).

Average particle size of emulsion III (E III) was 5.918 µm and of EVI – 3.352 µm, while the respective dispersion indexes amounted to 3.0 and 1.6 (Fig. 2). Microscope analysis affirmed these findings. The microscopic image exhibits individual

particles of greater diameters – recorded as additional fractions (Figs. 1, 2). Emulsion VI was characterized by a single fraction of uniformly homogenized particles, most likely caused by relatively high speed of the homogenization (24000 rpm). The low content of carboxymethyl cellulose in the emulsion (0.3%), however, obstructed skin application of the emulsion. The emulsion was rapidly absorbed without leaving any fatty deposits on the skin.

Emulsions I and II received the lowest sensory ratings regardless of the age group. The respondents claimed the preparations built a thick greasy film on their skins and produced a sense of viscous skin, treated as a hindrance to application of these preparations (Tables 3, 4). They were primarily characterized by a poor absorption, continuing for ca. 5 min. The foregoing observations were also confirmed by statistical analysis, demonstrating that the model emulsions, EI and EII, gained lower statistically significant ratings ($p < 0.05$). Morphologically, the systems EI and EII contained substantially larger particles than the remaining emulsions (Fig. 1). This was particularly true for emulsion II. Its particle distribution was uneven and included several areas with particles of varying diameters. Speeds of the homogenization process of both the emulsions were minimal (6000 rpm). This was likely to give rise to insufficient and inadequate homogenization. Application of different quantities of carboxymethyl cellulose, acting as a viscosity modifier in the system, in the process of emulsification did not improve properties of the systems. This is confirmed by the maximum value of average particle size (E I – 35.18 µm, E II – 74.35 µm) and a polydispersive particle distribution (Figs. 2, 3). The cumulative curve concerning emulsion I showed diameters of 90% balls to be below 40 µm, while 90% of emulsion II balls had diameters of less than 80 µm. The wide range of the droplets and their high average size are proof of a clearly unstable nature of the system (Fig. 3).

These results concerning stability of the systems tested are reaffirmed by results of two stability tests (temperature and centrifuge test). Emulsions IV and V passed both the tests successfully. The preparations, maintained at 3°C and then at a higher temperature of 40°C for 7 days, were homogeneous and without clotting at all times. They preserved their form until the end of the centrifuge experiment. Results for emulsions E III and E VI were satisfactory as well. However, these preparations broke on the fourth day of the testing, at 40°C, which was especially marked for emulsion VI. Clots and minor quantities of air bubbles were also noted. Regrettably, destabilizing changes were observed in both the systems after 20 min of the centrifuge testing. Emulsions E I and E II, on the other hand, failed all of the testing. Emulsion II broke completely as early as on the first day while E I did on the second day. Destabilizing changes caused by the centrifuge testing were observed as early as after the initial 10 min.

Corneometric testing showed skin moistening during the 90-min long testing to decline to a statistically significant degree ($p < 0.05$) in the first 15 min. The skin moistening levels varied at a clearly slower rate in the successive measurements. The skin moistening during 15-90 min was maximum on application of emulsion I - it rose by 37.6% in the younger group of respondents and by 46.8% for the other group of respondents on average with reference to the skin area where the preparation had not been applied (Figs. 4, 5). The skin moistening on application of emulsion E VI was minimum for both the age groups (Figs. 4, 5). Moistening with this preparation reached a mere 0.5% for females aged 18-35 after 90 min. On application of the remaining emulsions, E II – E V, variations of the moistening levels were comparable and within the range 22.1 - 26.6% for the age group 18-35 and 24.9-32.3% for the age group 40–50. With regard to the older grouping, E III and E IV exhibited a greater moistening

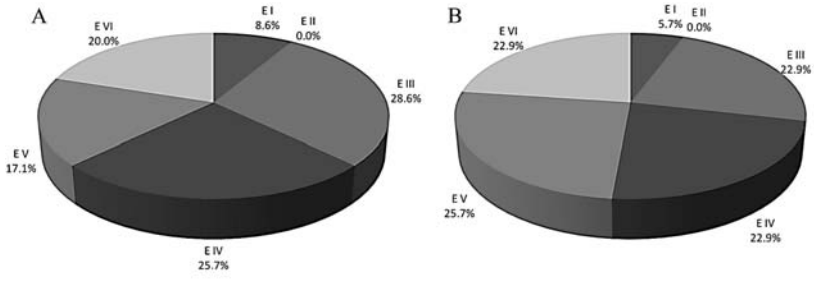

Figure 6. Percentage of people reporting the daily use of the emulsion: A - for the women aged 18-35 years, B - for the women aged 40-50 years

than emulsions II and V for the entire duration of measurements. In general, the emulsions applied were more effective in terms of the observed skin moistening for the group of females aged 40-50.

A great majority of those assessing the preparations showed maximum interest in E III, E IV and E V. Emulsion III was ultimately rated the best in the opinion of the youngest age group (28.6%), whereas the older respondent grouping chose emulsion V (25.7%) (Fig. 6 A, B).

CONCLUSIONS

Introduction to the systems tested of walnut oil triglycerides containing unsaturated acids and of mutton tallow triglycerides containing conjugated linoleic acid (CLA) is innovative and constitutes a valuable base for pharmaceutical or cosmetic emulsions. In addition, the preparations contained renewable emulsifiers that helped to stabilize the system. When all properties of the tested emulsions are taken into consideration, emulsion IV (containing 0.9 g carboxymethyl cellulose and homogenized at 18000 rpm) and emulsion V (1.5 g of carboxymethyl cellulose and homogenized at 24000 rpm) were found to be of optimum composition. The emulsions exhibited good stability, were highly rated in sensory terms and displayed optimum moistening properties. Emulsion III (containing 0.6 g carboxymethyl cellulose and homogenized at 12000 rpm) was also appreciated by the respondents, yet the average particle size and dispersion coefficient indicated the emulsion required more work on the recipe (enhanced revolutions at the time of homogenization or increased content of carboxymethyl cellulose). Emulsions III and IV demonstrated a constant moistening capacity in the entire period of measurement for the older female group. The younger respondents also said that emulsion III could be a preparation for everyday care. The older group of respondents chose emulsion V. The results led to the conclusion that addition of (0.6–1.5%) of carboxymethyl cellulose and the range of revolutions 12 000 do 24 000 rpm as part of the emulsion homogenization should produce emulsions of maximum stability and solid moistening and sensory properties. It has been proven that model emulsions based on interesterified fats containing renewable emulsifiers, with optimum carboxymethyl cellulose content and specific revolutions at the time of homogenization are an opportunity for developing preparations targeted at skins requiring special care (e.g., with atopic dermatitis or psoriasis). Results of testing of the six emulsions containing interesterified fat indicate that selection of such a fat base for a pharmaceutical or cosmetic preparation is reasonable and practicable. Generally, out results show that above mentioned emulsions are in most a natural effective preparations for improving skin hydration, possibly through a humectant mechanism, probably due to the presence of mutton tallow and walnut oil in fat blend. Consequently, they may be used as moisturizing formulations and also as a preparation in the treatment of dry skin. Besides, the work confirmed the possibility of using mutton tallow (by-product of meat industry) as one of the components of the fat phase of the emulsion.

REFERENCES

1. Kendall C.A., Nicolaou A.: Prog. Lipid Res. 52, 141 (2013).
2. Naik S., Basu A., Saikia R., Madan B., Paul P. et al.: J. Mol. Catal. B 65, 18 (2010).
3. Jenab E., Temelli F., Curtis J.M.: Food Chem. 141, 2220 (2013).
4. Tarnowska K., Kostecka M., Piotrkowicz A., Łobacz M., Kowalski B.: La Rivista Italiana Delle Sostanze Grasse 40, 95 (2013).
5. Marangoni A.G., Rousseau D.: Trends Food Sci. Tech. 6, 329 (1995).
6. Fredrick E.; Walstra P.; Dewettinck K.: Adv. Colloid Interfac. Sci. 153, 30 (2010).
7. Mohammad I.S., Khan H.M.S., Arshad A.I., Ijaz H., Banerjee P. et al.: Acta Pol. Pharm. Drug Res. 72, 1233 (2015).
8. Dickinson E.: Curr. Opin. Colloid Interfac. Sci. 15, 40 (2010).
9. Ramanauskiene K., Inkeniene A.M., Savickas A., Masteikova R., Brusokas V.A.: Acta Pol. Pharm. Drug Res. 66, 681 (2009).
10. Nazir H., Zhang W., Liu Y., Wang L., Naseer M.M., Ma G.: Int. J. Cosmetic Sci. 36, 124 (2014).
11. Chen B., McClements D.J., Decker E.A.: Food Chem. 142, 365-372 (2014).
12. Freitas L., Paula A.V., Dos Santos J.C., Zanin G.M., De Castro H.F.: J. Mol. Catal. B 65, 87 (2010).
13. Szeląg H., Pauzder B.: Colloid Surface A 219, 87 (2003).
14. Kowalska M., Zbikowska A., Kowalski B.: J. Am. Oil Chem. Soc. 91, 1703 (2014).
15. Adhikari P., Hu P., Yafei Z.: J. Am. Oil Chem. Soc. 89, 1961 (2012).
16. Lu D., Yang Y., Li Y., Sun C.: Curr. Pharm. Anal. 11, 66 (2015).

17. International Standards Official Methods 8420: Animal and Vegetable Fats and Oils – Determination of Polar Compounds Content. ISO, Geneva 2004.
18. Kleeman W.: Das Leder 11, 723 (1964).
19. Marzec A.: Cosmetic chemistry, (in Polish), 3rd edn., vol. 1. pp. 43-47; Scientific Society for Organization and Management Association of Higher Usability: Home Organizer, Toruń 2009,
20. Polańska A., Silny W., Jenerowicz D., Knioła K., Molińska-Glura M. et al.: Skin Res. Technol. 21, 35 (2015).
21. Martini M.C.: Cosmetology and Skin Pharmacology, (in Polish). 1st edn., vol. 1, pp. 37-52, Medical Publisher PZWL, Warsaw 2009.
22. Bojanowicz H., Woźniak B.: Probl. Hig. Epidem. 89, 471 (2008) (in Polish).
23. Horrobin D.F.: Am. J. Clin. Nutr. 71, 367 (2000).

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