

1 **EMULSIONS BASED ON FATTY ACID FROM VEGETABLE OILS FOR COSMETICS**

2 Oleksandra Kunik^{a*}, Diana Saribekova^a, Giuseppe Lazzara^b, Giuseppe Cavallaro^b

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4 ^a Department of Chemical Technologies, Expertise and Food Safety, Faculty of Integrated
5 Technologies, Kherson National Technical University, st. Beryslavskoe shose, 24, 73008, Kherson,
6 Ukraine.

7 ^b Department of Physics and Chemistry, University of Palermo, Viale delle scienze pad 17, 90128,
8 Palermo, Italy.

9 *Corresponding author: Oleksandra Kunik e-mail: kunyk.oleksandra@kntu.net.ua

10
11 **Abstract**

12 Vegetable oils are sources of saturated, monounsaturated and polyunsaturated fatty acids. In this
13 work, we studied the suitability of a mixture of vegetable oils and some fatty acids (similar to the those
14 of the human skin) as a cosmetic emulsion. Hypericum (*Hypericum perforatum L.*) – 1%, thistle
15 (*Silybum marianum (L.) Gaertn.*) – 42%, linen (*Linum usitatissimum L.*) – 1.1%, wheat germ (*Triticum*
16 *aestivum L.*) – 1%, sesame (*Sésamum indicum L.*) – 1%, mustard (*Sinápis álba L.*) – 2%, pumpkin
17 (*Cucúrbita pépo L.*) – 5%, – were selected as vegetable oils. The emulsion based on the proposed
18 mixture of vegetable oils has a higher antioxidant activity (1.623 OD) compared with that based on
19 mineral oil (0.427 OD). Finally, the emulsion provides a good balance of moisture and fat on the skin
20 for one hour, in contrast to the short-term effect of the emulsion based on mineral oil. The obtained
21 data are promising for the formulation of emulsion with specific fatty acids composition.

22
23 **Keywords:** vegetable oils, fatty acids, emulsion, antioxidant activity, moisture, skin.

1. Introduction

On the territory of modern Ukraine the steppe is 240 thousand km², or 40% of the entire territory of the country, and is the largest zonal natural complex (Pirko, 2004). The steppe is an area where in dry conditions develops the largest variety of herbaceous plants on the planet, which begin to bloom immediately after the snow melts, from the beginning of February. If in the forest there are from 2 to 5 types of plants per 1 m², then in the steppe – more than 100 (Vasylyuk, 2021). Crop production in the steppe zone specializes in the production of winter wheat, barley, corn, soybeans, sunflower seeds, rapeseed, and the cultivation of vegetable crops (Bazalii et al., 2015). Vegetable raw materials have been recently used for numerous applications, including paper fabrication (Modica et al., 2020; Sottile et al., 2021), synthesis of anti-inflammatory products (Maione et al., 2016), protection of artworks (D'Agostino et al., 2021) and cosmetic emulsions (Chu and Nyam, 2020; Saribekova et al., 2021; Khafid et al., 2022). Among the natural materials, fatty vegetable oils (triglyceride) are valuable components of medicinal and decorative cosmetics. As a part of cosmetic compositions vegetable oils carry out, as a rule, some functions. They play the role of emollients, serve as a base in which other structural and functional components of the composition are located, perform a transport function of delivery of biologically active substances through the lipid barrier of the skin. The triglycerides of fatty acids, which form the basis of vegetable oils, are actively involved in the formation of structure, function and repair of cell membranes of the lipid barrier. Triglycerides of polyunsaturated fatty acids play an important role in the skin's immune system, participating in the synthesis of prostaglandins (Shepel, 2010; Hernandez and Margolina, 2017).

In determining the composition of fatty acids (triglycerides), which would provide the most positive effect of a particular cosmetic product, it is advisable to use triglycerides that are part of the skin's lipid barrier in a normal healthy skin considering also their natural proportions (Shepel, 2010). Fatty acids, which are naturally present in the human epidermis, are mainly a mixture of linoleic C18:2 (21.5%), oleic C18:1 (15.1%), palmitic C16:0 (14.0%), stearic C18:0 (11.1%) and lignoceric C24:0 (10.0%)

acids, Fig. 1 (Moore et al., 2020; Knox and O’Boyle, 2021). Acids such as arachidonic C20:4 (6.2%), behenic C22:0 (2.7%), palmitinoleic C16:1 (2.3%), arachinoic C20:0 (1.6%), myristic C14:0 (1.1%), linolenic C18:3 (1.0%) are present in much smaller amounts but still play a key role in a healthy skin.

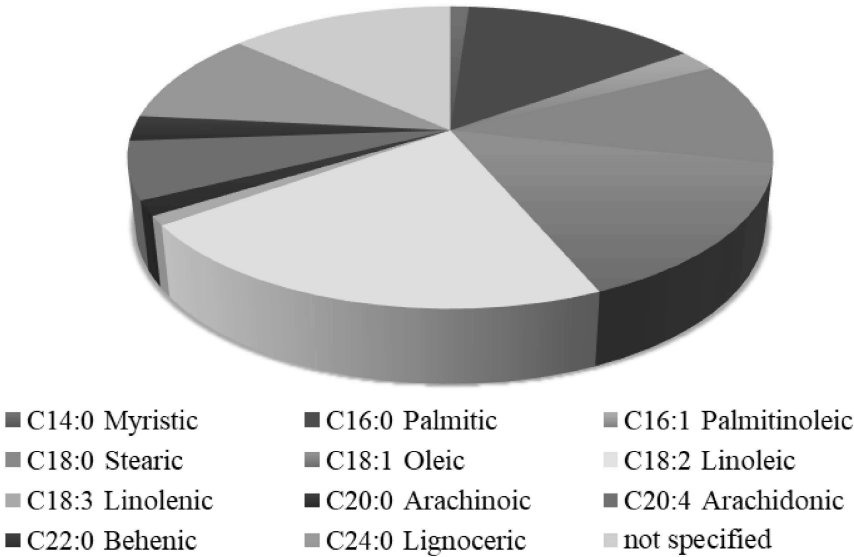


Figure 1. The average percentage of fatty acids in the epidermis of normal human skin.

It is reported (Shepel, 2010) that the mixture of oils, close in fatty acid composition of normal skin, was palmitic (2 – 3%), palmitoleic (13 – 17%), stearic (1 – 2%), oleic (30 – 36%), linoleic (20 – 26%) and linolenic (2 – 3%) for a cosmetic formulation. In addition, it is emphasizes the importance of maintaining the ratio of linoleic/oleic 1:1.8 and linoleic/linolenic acids 10:1. This quantitative ratio of linoleic/oleic and linoleic/linolenic acids is also reported by other authors (Nosenko et al., 2015, Mank and Polonska, 2016). Finally, the optimal ratio between saturated, mono- and polyunsaturated fatty acids is 1:1:1 when designing compositions of natural oils, which corresponds to that of lipid layers of the skin barrier layer (Mank and Polonska, 2016).

Recent literature reports slightly different values from the fatty acid composition of epidermis in normal skin (Moore et al., 2020; Knox and O’Boyle, 2021).

The more recent tendency is to select the composition of natural vegetable oils according to the fatty acid composition of the sebum of normal skin. Such a calculated mixture of oils should ensure the balance of moisture and fat on the skin treatment, as well as the antioxidant properties due to the presence of biologically active compounds in vegetable oils. As follows, the aim of the work is to develop a mixture of vegetable oils from the steppe zone of southern Ukraine with a fatty acid composition as close as possible to the fatty acid composition of the epidermis of normal skin for use as a fat phase in cosmetic emulsions.

2. Materials and Methods

2.1. Materials

2.1.1. Unrefined vegetable oil

In the work, we investigated samples of the unrefined vegetable oils typical of the steppe zone of southern Ukraine obtained by the press method: samples of oil of brier (*Rosa cinnamomea sensu L.*), sunflower (*Helianthus annuus L.*), wheat germ (*Triticum aestivum L.*), thistle (*Silybum marianum (L.) Gaertn.*), grape seeds (*Vitis vinifera L.*), sesame (*Sésamum indicum L.*), sea-buckthorn (*Hippóphaë rhamnóides L.*), camelina (*Camelina sativa (L.) Crantz*), linen (*Línium usitatíssimum L.*), pumpkin (*Cucúrbita pépo L.*), mustard (*Sinápis álba L.*), hemp (*Cánnabis sativa L.*); and method of extraction by oil corn (*Zéa máys L.*) refined deodorized: samples of oil of hypericum (*Hypericum perforatum L.*), chamomile (*Matricaria chamomilla L.*) and bidens (*Bídens tripartíta L.*), supplier company Leko Style (Ukraine).

2.1.2. Components of the cosmetic emulsion

Emulsifier – non-ionic emulsion system Emulgade SE-PF (INCI: Glyceryl Stearate (and) Ceteareth-20 (and) Ceteareth-12 (and) Cetearyl Alcohol (and) Cetyl Palmitate) – BASF SE, Germany; structuring

92 – cetearyl alcohol (INCI: Cetearyl Alcohol) – BASF SE, Germany; conservative – Cosgard (INCI:
93 Benzyl alcohol, Dehydroacetic acid, Aqua) – Lonza, Switzerland; pH regulator – citric acid (INCI:
94 Citric acid) – Nanjing Jiayi Sunway Chemical Co., Ltd, China; silicone – dimethicone (INCI:
95 Dimethicone) – BRB International BV, Netherlands.

96

97 *2.2. Method of research of unrefined vegetable oils*

98

99 *2.2.1. Fatty acid composition of oils*

100 The study was performed on a gas chromatograph Crystal-2000M, Agilent DB-FFAP column 50 m ×
101 0.320 mm × 0.50 μm (Kuryata and Polyvanyi, 2018).

102 *2.2.2. Refractive index*

103 The determination of the refractive index of unrefined vegetable oils was performed using the
104 refractometric method (Bhagyaraj et al., 2020). To determine the refractive index of unrefined
105 vegetable oils, a refractometer type IRF-22 was used.

106 *2.2.3. Color number on the iodine scale*

107 The color of the test oil was evaluated by comparison with the color of standard iodine solutions in
108 the range from 1 to 100 mg of iodine (Shimamoto et al., 2016).

109 *2.2.4. Determination of acid number by titrometric method*

110 A portion of the test oil (emulsion) was added to a 250 mL conical flask. Add 50 mL of neutralized
111 solvent mixture (diethyl ether and ethyl alcohol) and mix. The resulting solution was rapidly titrated
112 with potassium or sodium hydroxide solution until a pale pink color persisted for 15 s. The acid number
113 (mg KOH/g) was calculated by the formula:

$$AN = \frac{V \cdot c \cdot 56.1}{m}, \quad (1)$$

114 where c is the exact value of the molar concentration of the alkaline titrant, mol/1000 mL; V is the
115 volume of alkaline titrant determined during indicator titration or calculated for the equivalent point
116 during potentiometric titration, mL; 56.1 is the molar mass of potassium hydroxide, g/mol; m is the
117 amount of oil (emulsion), g (Aricetti and Tubino, 2012).

118

119 2.3. *Emulsion*

120

121 2.3.1. *Preparation of the emulsion*

122 To prepare the emulsion, oil, cetearyl alcohol and Emulgade SE-PF were heated in a glass in a water
123 bath at 60°C. In parallel, the calculated amount of water and citric acid was heated in the second glass
124 in a water bath to the same temperature (60°C). Both phases were mixed at a speed of 200 min⁻¹ for 5
125 min. The temperature of the mixture was periodically measured during the emulsification process.
126 After cooling the emulsion to 30°C was introduced conservative Cosgard, stirring was continued for 5
127 min.

128 2.3.2. *Emulsion colloidal stability*

129 The glass tubes were filled to 2/3 of the volume with the test emulsion, placed in a thermostat and
130 kept at 42°C for 20 min. The height of the test tubes is 120 mm, the diameter is 14 mm. After that, the
131 tubes were centrifuged at a speed of 100 s⁻¹ for 5 min. The emulsion was considered stable if no more
132 than a drop of the aqueous phase or a layer of the oil phase of not more than 0.5 cm was isolated in the
133 test tubes after centrifugation (Estanqueiro et al., 2014).

134 2.3.3. *Determination of emulsion thermal stability*

135 Tubes with a capacity of 25 mL were filled to 2/3 of the volume with the tested emulsion, making
136 sure that no air bubbles remained in them, closed with stoppers and placed in a thermostat with a
137 temperature of 40 – 42°C. The contents of the tubes after 1 hour were gently stirred with a glass rod to
138 remove air. The emulsions were kept in a thermostat for 24 hours. The emulsion was considered stable

139 if no aqueous phase evolution was observed in the tubes after thermostating, and an oil phase layer of
140 no more than 0.5 cm is allowed to be released (Estanqueiro et al., 2014).

141 2.3.4. *Determination of hydrogen index*

142 The determination was performed in an aqueous extract using a pH meter ADWA AD1200. To
143 prepare an aqueous extract of the emulsion, 10.00 g of the sample was placed in a beaker, 90 mL of
144 distilled water was added, heated with stirring to 70°C until the emulsion was completely destroyed
145 (separation of the oil layer), cooled to 25°C and the aqueous layer separated.

146 2.3.5. *Determining the amount of moisture and fat on the skin*

147 The amount of moisture and fat was determined using a tester with a digital high-sensitivity sensor
148 Skin Detector SG-7D. The study involved 60 youth volunteers (22 – 35 years old), including 30 males
149 and 30 females during autumn.

150 2.3.6. *Antioxidant activity*

151 The antioxidant activity of the cosmetic emulsion was determined using the Oyaizu method (FRAP
152 method) [Faria et al., 2005; Harhaun et al., 2020]. A total of 0.2 mL of the sample was mixed with
153 phosphate buffer (0.5 mL; 0.2 M; and pH 6.6) and potassium ferricyanide [$K_3Fe(CN)_6$] (0.5 mL; 1%
154 solution). The resulting mixture was kept at 50°C for 20 min, then 0.5 mL of a 30% solution of
155 trichloroacetic acid was added to the mixture and filtered. To 0.5 mL of the obtained filtrate was added
156 ferric (III) chloride $FeCl_3$ (0.1 mL, 0.1% solution). The optical density was determined using a ULAB
157 102 spectrophotometer (Shanghai Metash Instruments Co., Ltd., Shanghai, China) at a wavelength of
158 700 nm. The increase in the absorption of the reaction mixture indicates an increase in the ability of the
159 samples to reduce ferric iron ($Fe^{3+} \rightarrow Fe^{2+}$). A 20% solution of ascorbic acid was used as a reference
160 solution.

161

162 2.4. *Statistical processing of the obtained results*

163

164 The software Origin 6.1 (OriginLab Corporation, UK) was used for statistical processing of the
165 obtained results. The level of statistical significance is $p<0.05$.

166

167 **3. Results and discussion**

168 At the first stage of the study, the characteristics of the studied samples of vegetable oils were
169 determined: refractive index, acid number and color number on the iodine scale (Table 1).

170 **Table 1.** Characteristics of the studied samples of vegetable oils

Oil	Refractive index	Acid number, mg KOH/g	Color number on the iodine scale
Brier	1.475	0.8	70
Sunflower	1.473	0.3	15
Wheat germ	1.471	0.3	25
Thistle	1.471	0.7	10
Grape seeds	1.474	0.7	20
Sesame	1.472	0.4	5
Bidens	1.474	0.4	10
Sea buckthorn	1.441	1.6	Over 100
Chamomile	1.474	0.4	5
Hypericum	1.473	0.4	25
Camelina	1.476	0.9	40
Linen	1.481	2.2	40
Pumpkin	1.473	0.5	50
Mustard	1.474	0.8	25
Hemp	1.476	1.4	20

171

172 According to Table 1, the studied samples of vegetable oils are identical in terms of refraction
173 (Gunstone, 2004; Mukhametov et al., 2022). The acid number corresponds to the indicators specified in
174 the quality certificates of vegetable oil. Analysis of the color number shows that the most colored are
175 samples of sea buckthorn oil – more than 100 units and brier oil – 70 units of iodine scale, whilst the
176 least colored were samples of sesame oil and chamomile – 5 units of iodine scale. The data obtained
177 should be taken into account when creating a composition of vegetable oils due to possible undesirable
178 color (Sharma et al., 2022).

179 The fatty acid composition of the studied samples of vegetable oil is provided in Table 2.

Table 2. Fatty acid mass percentage composition of vegetable oils

Oil													
Fatty acid	brier	sunflower	wheat germ	thistle	grape seeds	sesame	bidens	sea buckthorn	chamomile	hypericum	camelina	linen	pumpkin
C14:0 ^a	0.070	0.064	0.075	0.085	0.070	0.021	0.074	0.078	0.072	0.070	0.070	0.049	0.107
C15:0				0.036				0.015			0.025	0.021	
C16:0	6.413	6.340	6.630	7.088	6.694	9.448	6.563	6.687	6.569	6.455	6.170	6.130	9.455
C16:1	0.122	0.122	0.147	0.101	0.147	0.160	0.128	0.132	0.107	0.125	0.160	0.120	0.134
C17:0	0.045	0.037	0.038	0.071	0.043	0.053	0.038	0.039	0.038	0.034		0.068	0.096
C17:1	0.024	0.022	0.024	0.040	0.027	0.027	0.021	0.024	0.021	0.019	0.034	0.035	0.046
C18:0	3.545	3.416	2.998	5.786	3.286	5.403	3.201	3.125	3.356	3.395	2.735	5.407	4.900
C18:1	15.165	30.097	29.384	38.073	27.906	40.536	28.716	26.369	27.670	26.238	17.192	21.243	31.645
C18:2	42.974	58.465	58.982	39.402	60.282	43.038	59.596	61.915	60.111	61.992	22.750	15.226	52.052
C18:3	30.074	0.099	0.151	0.949	0.334	0.283	0.179	0.075	0.259	0.094	28.845	51.094	0.224
C20:0	0.242	0.256	0.236	3.724	0.222	0.558	0.271	0.239	0.363	0.247	1.666	0.187	0.381
C20:1	0.190	0.164	0.181	1.391	0.170	0.179	0.241	0.195	0.324	0.202	13.210	0.139	0.137
C20:2				0.070							1.587		
C22:0	0.756	0.681	0.698	2.480	0.583	0.188	0.718	0.703	0.776	0.707	0.404	0.182	0.450
C22:1	0.031		0.036										0.040
C22:2	0.102		0.116					0.131	0.075				0.198
C24:0	0.021		0.036										
C24:1	0.230	0.238	0.269	0.705	0.234	0.105	0.254	0.274	0.259	0.259	0.228	0.098	0.135

^a The blue color indicates the fatty acids found in normal skin.

In the analysis of the fatty acid composition of the studied samples of vegetable oil, the main attention was paid to the fatty acids of normal skin (Fig. 1): C14: 0, C16: 0, C16: 1, C18: 0, C18: 1, C18: 2, C18: 3, C20: 0, C22: 0, C24: 0. Based on the data in Table 2, samples of oils with the maximum content of fatty acids detected in normal skin were identified (Table 3).

Table 3. Samples of oils with the maximum content of certain fatty acids

Fatty acid	Oil	Value, %
Myristic C14:0	Pumpkin	0.107
Palmitic C16:0	Pumpkin	9.455
Palmitinoleic C16:1	Mustard	0.172
Stearic C18:0	Thistle	5.786
Oleic C18:1	Sesame	40.536
Linoleic C18:2	Hypericum	61.992
Linolenic C18:3	Linen	51.094
Arachinoic C20:0	Thistle	3.724
Arachidonic C20:4	—	—
Behenic C22:0	Thistle	2.480
Lignoceric C24:0	Wheat germ	0.036

Therefore one can conclude that among the studied samples of vegetable oil, the following should be selected for the proper skin treatment formulation: pumpkin, mustard, thistle, sesame, hypericum, linen and wheat germ. It should be noted that arachidonic acid C20:4 was not detected in any of the studied samples of vegetable oil (Vicentini-Polette et al., 2021). The percentage of selected vegetable oils was calculated, which in the ratio of essential fatty acids is as close as possible to the ratio of fatty acids in normal healthy skin (Table 4).

Table 4. Estimated composition of basic fatty acids of the calculated mixture

Oil	Concentration, %
Hypericum	1.0
Thistle	42.0
Linen	1.1
Wheat germ	1.0
Sesame	1.0
Mustard	2.0
Pumpkin	5.0

The fatty acid composition of normal skin (a) and the calculated mixture of oils (b) are compared in Fig. 2.

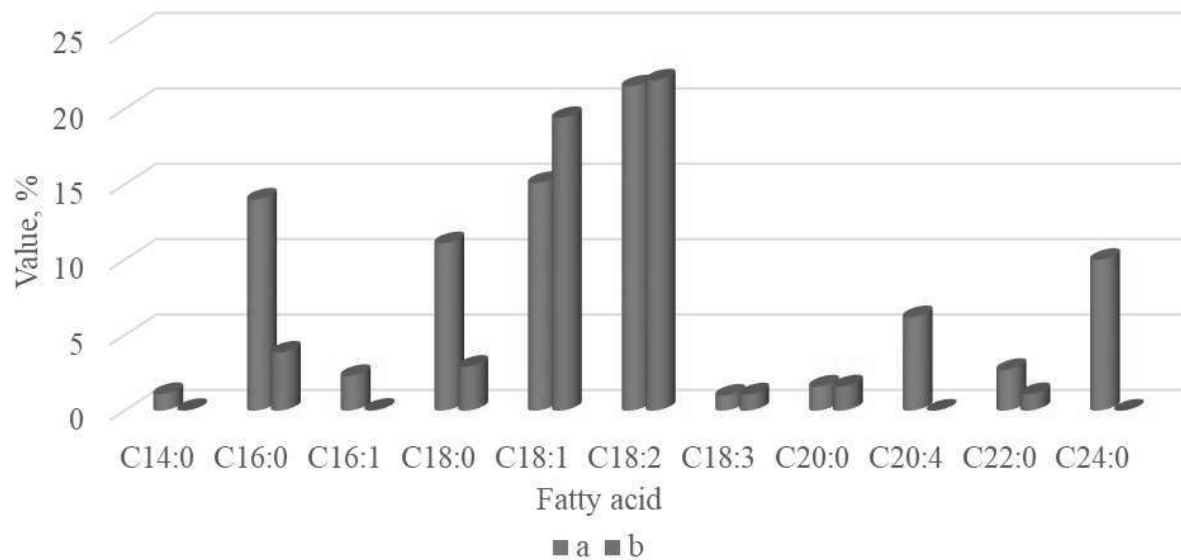


Figure 2. Fatty acid composition: a – normal skin, b – calculated mixture of vegetable oils.

The calculated mixture of vegetable oils does not contain arachidonic acid C20:4 (Figure 2). Also the content of fatty acids such as myristic C14:0, palmitic C16:0, palmitinoic C16:1, stearic C18:0, behenic C22:0 and lignoceric C24:0 is relatively small (Figure 2). Optimal values have been achieved for acids such as oleic C18:1, linoleic C18:2, linolenic C18:3 and arachinoic C20:0 (Figure 2).

For the sake of comparison, we developed mixture of vegetable oils, – Emulsion I and a similar composition with the replacement of the mixture of oils with mineral oil – Emulsion II (Table 5). The Emulsion II was proposed by us in a recent paper (Kunik et al., 2019).

Table 5. Prescription composition of emulsion cosmetics

Component	Concentration, %	
	Emulsion I	Emulsion II
Mixture of vegetable oils	25.0	–
Mineral oil	–	25.0
Emulgade SE-PF	3.0	
Cetearyl alcohol	3.0	
Dimethicone	3.0	
Cosgard	0.5	
Citric acid	To pH 5.5	
Distilled water	To 100	

The obtained samples of the emulsion were investigated to determine the organoleptic, physical and chemical characteristics (Table 6).

Table 6. Organoleptic, physical and chemical characteristics of the obtained samples of emulsions

Indicator	Emulsion I	Emulsion II
Appearance	Homogeneous emulsion, without impurities	
Color	Yellow	White
Scent	Characteristic scent of vegetable oils	Neutral
Colloidal stability	Stable	
Thermal stability	Stable	
Hydrogen index (pH)	5.5	
Antioxidant activity, optical density D	1.623	0.427

The obtained data show that the obtained emulsions are stable colloidal system. The antioxidant activity of the emulsion I significantly exceeds the antioxidant activity of the Emulsion II based on mineral oil. The obtained data are consistent with (Saha and Ghosh, 2011).

Next, we determined the effect of the developed composition of the cosmetic emulsion on the amount of moisture and fat on the skin of the forearm (Table 7).

225 **Table 7.** Determining the effect of moisture and fat on the skin

Time	Indicators of skin		Cosmetic emulsion based		Cosmetic emulsion	
	without cosmetic		on mineral oil		based on unrefined	
	emulsion				vegetable oils	
	moisture, %	fat, %	moisture, %	fat, %	moisture, %	fat, %
Before application	21.0/20.4 ^a	31.3/33.5 ^c	21.0/20.4	31.1/33.1	21.1/20.4	31.0/32.9
Immediately after application	20.9/20.0 ^b	30.5/32.7	48.4/45.9	32.5/35.3	52.9/50.3	38.5/40.3
After 1 hour	21.3/19.8	31.9/33.4	25.2/21.7	33.6/36.1	35.6/33.4	44.4/45.2
After 2 hours	20.8/20.1	31.2/33.2	21.5/20.8	32.0/33.2	29.8/26.5	37.8/39.6
After 3 hours	21.0/20.0	31.2/33.5	21.2/20.5	31.8/33.9	25.2/23.0	38.7/39.2
After 4 hours	21.2/20.1	31.1/33.0	21.0/20.3	31.6/33.2	20.7/19.5	31.0/33.8

226 ^a The average amount of moisture and fat on the skin of female and male volunteers.

227 ^b The red color indicates an imbalance of moisture and fat on the skin, green – the balance.

228 ^c The level of statistical significance is $p < 0.05$.

229

230 According to Table 7, immediately after applying both emulsions on the skin of volunteers, a

231 balance of moisture and fat was observed. An hour later, the balance of moisture and fat was

232 observed only after applying a sample of emulsion I. After two hours, the balance of moisture

233 and fat was lost, although the moisture and fat content of the skin after applying the emulsion I

234 was higher than after applying the emulsion II with mineral oil. Three hours after the application

of emulsion I, the moisture and fat content of the skin was still 20 – 24% higher than the moisture and fat content of the skin without applying the emulsion. The indicators of moisture and fat content on the skin did not differ from the indicators of skin without emulsion application after 4 hours from application. It should be noted that with the systematic application of the developed composition of the emulsion with a mixture of oils, it is possible to expect a longer effect of the balance of moisture and fat on the skin.

The results also show that women's skin, unlike men's, is more moisturized and less oily. The obtained data are fully consistent with literature reports (Patel et al., 2017; Rahrovan et al., 2018).

This work proves the perspective of taking into account the composition of fatty acids in the formulation of emulsions for cosmetic purposes. Further research will be aimed at prolonging the moisturizing effect by including in the mixture of vegetable oils rich in myristic C14:0, palmitic C16:0, palmitoleic C16:1, stearic C18:0, arachidonic C20:4 and lignoceric C24:0 fatty acids.

4. Conclusions

Selected samples of vegetable oil from steppe zone of southern Ukraine were characterized from the physico-chemical point of view (refractive index, color and acid number). And also by determining the fatty acid composition. A formulation for cosmetic application was prepared by considering the ratio of fatty acids as close as possible to that in healthy skin. An additional emulsion formulation containing mineral oil was considered and the performances were compared to the vegetable oil based one. It was demonstrated that the vegetable oil based emulsion has higher antioxidant activity and it provides a balance of moisture and fat on the skin for longer time compared to the emulsion based on mineral oil.

Declaration of Interest statement

No potential conflict of interest was reported by the authors.

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