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Conference paper

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Formulation and characterization of some oil in water cosmetic emulsions based on collagen hydrolysate and vegetable oils mixtures

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Abstract: The present study was focused on the preparation and characterization of different topical oil in water emulsions based on natural ingredients such as collagen hydrolysate and vegetable oils mixtures. As oil phases, mixtures of coconut, almond, jojoba, avocado oils (in different ratios) were used while the water phase consisted in lavender water. As emulsifier, a plant based product containing glyceryl stearate and potassium stearate was incorporated (in the concentration range 4–5 %), while as rheology modifier xanthan gum was used (in the concentration range 0.2–1 %). As preservatives, Cosgard (containing benzyl alcohol, salicylic acid, glycerol, ascorbic acid) and mixtures of two essential oils (lemon and lavender) were selected. The designed formulations were assessed by means of their physico-chemical properties (pH, viscosity, thixotropic behavior), optical microscopy analysis and sensorial characteristics. The obtained emulsions were found to present pseudoplastic and thixotropic behaviors, adequate for cosmetic skin care product formulations.

Keywords: Chemistry for Beauty and Health 2018; O/W cosmetic emulsions; rheology; thixotropy; vegetable oils.

Introduction

The preservation of skin health and the skin disorders that affect the life quality of many patients have been the object of several studies on the preparation and characterization of a wide range of skin-care cosmetics and dermatocosmetic products. The main raw materials used to obtain cosmetics are water, oily materials (oils, fats), humectants, antioxidants, preservatives, pharmaceutical agents, fragrances [1]. An important class of cosmetic products is represented by creams. They must contain mainly occlusive,

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humectant and emollient ingredients [2]. The occlusive ingredients physically block transepidermal water loss by forming a hydrophobic film on the skin surface and within the superficial interstitium between corneocytes [3]. Common occlusive substances in moisturizers are lipids (petrolatum, beeswax, lanolin and various oils) [4]. The humectants attract water from dermis to epidermis. Most of them are represented by low molecular weight substances such as glycerol (probably the most commonly used humectant), urea, propylene glycol and pyrrolidone carboxylic acid [5], α-hydroxy acids (lactic, glycolic and tartaric acids); few high molecular weight substances such as hyaluronic acid, are also used in moisturizers. The emollients smooth skin by filling the space between skin flakes with a droplet of oil [6]. Common fats and oils used as emollients for moisturizers are mineral oils, waxes, long-chain esters, fatty acids, lanolin and mono-, di- and triglycerides [7].

Nowadays, there is a growing interest in skin cosmetic and dermatocosmetic products containing natural ingredients, such as: vegetable and/or essential oils and collagen hydrolysate [8]. Cosmetic emulsions based on vegetable oils (VO) are the subject of many studies reported in the literature. VOs are preferred instead of mineral oils, due to their characteristics: biocompatibility and biodegradability, effectiveness in protecting the skin against solar radiation, inflammation, insect attack, microorganisms, and viruses [9]. The complex mixtures of bioactive compounds in the herbal ingredients are devoid of side effects commonly seen in the case of synthetic products. They are also characterized by low toxicity, cleansing ability, emulsification, moisturization, skin appearance, feel, fragrance, and lubrication. Moreover, in comparison to mineral oils, vegetable oils exhibit low viscosity and molecular weight, which makes them less occlusive (Silva, Soares, 1996) [10]. The vegetable oils contain as major compounds (95–98 %), triglycerides (esters of glycerol with three fatty acid molecules). The minor components in vegetable oils (less than 5 %: fatty alcohols, phytosterols, vitamins, fatty acids) are also of interest for their interesting biological properties being used in pharmaceutical and nutraceutical industries [11]. The oil uses for cosmetic purpose depend mainly on the type and proportion of fatty acids which vary according to the species of plants. According to the saturation degree of fatty acids, they can be classified into saturated, mono- and poly-unsaturated fatty acids. The fatty acids oleic, lauric, palmitic, myristic, and stearic acids were used as base components (of the oil phase) of many cosmetic formulations [12]. Oils such as almond oil, apricot oil, avocado oil, soybean oil, cocoa oil and palm oil have high content of unsaturated fatty acids such as oleic, linoleic and linolenic acid [13]. The strongest cosmetic effect was usually found for unsaturated fatty acids, especially ω-3 and ω-6. In skin care, the most important oils are those with a high content of linoleic acid (ω-6) and α-linoleic acid, as they are the least comedogenic and combat the occurrence of eczema [14]. Highly saturated vegetable oils are also important ingredients in the cosmetic and pharmaceutical formulations due to their fewer tendencies to produce free radicals from the oxidation process. As example, coconut oil was found to be suitable for the preparation of cosmetic due to high composition of saturated fatty acids (lauric, 50 % and myristic, 20 %) and minor content of caprylic acid that exhibited antifungal activity [15]. Vegetable oils with different degree of saturation levels (palm olein (POo), olive oil (OO), sunflower oil (SAF), grape seed oil (GSO), soybean oil (SBO) and sunflower oil (SFO)) were used as major ingredient of oil phases, their effects on emulsion stability being evaluated [16]. Emulsification of vegetable oils (sunflower oil, virgin coconut oil, extra virgin olive oil [17]; sesame oils [18]; hemp oil [19]) with different non-ionic surfactants were prepared and characterized. The development of products with high amounts of plant components led to their standardization. Organic Monitor (a research and consulting company specialized in global organic and related product industries), finds there are currently over 30 standards for cosmetics and personal care products, with most in Europe [20, 21]. Also, according to the latest statistics from the journal Organic Monitor natural and organic cosmetics account for 3 % of all cosmetic sales in Europe and their market share increased by 20 % a year. Botanical cosmetic ingredients include vegetable oils [22]. In skin cosmetic products, essential oils (EOs) obtained from various plants (tea tree, thyme, lemon grass, oregano, rosemary, calamint, lavender and many others) are also extensively used proving antimicrobial activities. A previous study showed that the antimicrobial effect of EOs mixtures depends on content, concentration and interactions between their active ingredients, leading to synergistic properties [23]. The essential oils have also been proposed as natural preservatives [24]. Moreover, the synergistic effect of synthetic preservative with an addition of essential oils in cosmetic preparations was reported [25]. Another important component with a great potential for use in the skin cosmetic formulations was reported to be collagen hydrolysate [26]. It was reported that collagen has a double action in the skin. On one hand, collagen hydrolysate provides the building block for elastin and collagen formation and, on the other hand, it act as ligands or binding receptors in fibroblasts to stimulate the above-mentioned components and hyaluronic acid [27, 28]. It was also demonstrated that collagen hydrolysate exerts antioxidant properties, reparative properties, antihypertensive activity and lipid-lowering activity in damaged skin [29, 30]. Collagen is also selected for use in cosmetic industry due to its biodegradability, availability and biocompatibility [31], being a good surface-active agent and demonstrating its ability to penetrate a lipid-free interface [32]. Also, it was recognized to be an effective natural humectant [33].

The aim of this study was to prepare different oil in water skin cosmetic emulsions based primarily on natural ingredients such as collagen hydrolysate, vegetable oils mixtures (avocado, jojoba, coconut and almond oils), a mixture of lavender and lemon essential oils and a plant based emulsifier containing glyceryl stearate and potassium stearate. The emulsions were evaluated by means of physicochemical parameters (pH, viscosity and level of thixotropy), based on their sensory properties and by optical microscopy analysis.

Experimental

Materials

Vegetable oils (coconut, almond, jojoba, avocado), lavender water, distilled water, xanthan gum, allantoin, coconut powder, Bella emulsifier (a plant based emulsifier containing glyceryl stearate and potassium stearate), Cosgard preservative (containing benzyl alcohol, salicylic acid, glycerol, ascorbic acid) were purchased from a local pharmacy. All these ingredients are delivered by Elemental SRL, Romania. All ingredients were reagent-grade chemicals. Collagen hydrolysate was obtained by acid hydrolysis of wet white leather wastes at 125 °C, for 8 h, according to the previously described technology [34]. Lemon and lavender essential oils were obtained by classical hydrodistillation using Neo-Clevenger apparatus as we described in our previous study [23].

Methods

Formulation of oil/water (O/W) emulsions

All the ingredients were carefully selected and used for the emulsion formulations according to the rules provided by The European Cosmetics Legislation [35].

The compositions of the prepared cosmetic emulsions are shown in Table 1.

Preparation of emulsions

The emulsions were prepared using a domestic blender [Homogenizer (Euro-Star, IKA D 230, Germany)]. The ingredients of phase A and phase B were individually heated in water baths into Berzelius beakers, periodically homogenizing the composition. When both phases reached a temperature of about 70–75 °C, they were removed from the water bath; then, the content of phase B was slowly added over phase A by mixing constantly. The mixing process continued for few minutes, avoiding as much as possible the emulsion aeration. The ingredients from phase C were added to the mixture and then dispersed for a few minutes, using

Table 1: Composition of the prepared cosmetic emulsions.

Phase	Ingredient	Percentage by weight				
		1	2	3	4	5
A	Coconut oil	10	10	20	20	10
	Almond oil	10	10	10	10	5
	Jojoba oil	10	5	0	5	3
	Avocado oil	10	5	0	5	3
	Bella emulsifier[a]	5	5	5	5	4
B	Distilled water	25	30	30	25	35
	Lavender water	25	30	30	25	35
C	Collagen hydrolysate	1	1	1	1	1
	Xanthan gum	1	0.5	0.2	0.2	0.2
	Allantoin	1	1.5	1.8	1.8	1.5
	Coconut powder	1	1	1	1	1.3
D1	Cosgard preservative	0.5	0.5	0.5	0.5	0.5
D2	Lemon and lavender essential oils mixture (1:1)	0.5	0.5	0.5	0.5	0.5

A, Oil phase; B, water phase; C, ingredients in solid phase; D1, preservative and heat-sensitive ingredient (Cosgard: mixture of benzyl alcohol, salicylic acid, glycerin, ascorbic acid); D2, preservative and fragrance. [a]A natural plant based O/W emulsifier containing glyceryl stearate and potassium stearate.

the blender, until a homogenous composition was achieved. The beaker was placed in a cold water glass and the composition was continuously stirred. When the temperature was decreased to 30 °C, the ingredients of phase D were added in the composition and mixed slowly. The temperature of essential oils addition in the composition is very important as it influences the amount of essential oils in the environment. A study pointed out that essential oils should be added at temperatures under 40 °C in cosmetic compositions and the amount of essential oils in the environment decreased as environmental temperature increased [36]. The obtained emulsions were cooled to room temperature under slow and continuous stirring, then transferred to eco-friendly and odorless sterile containers, until used.

Calculation of required hydrophilic–lipophilic balance values (*rHLB*) for the prepared emulsifiers

Required HLB value represents the *HLB* value required to prepare stable emulsion using combination of emulsifiers. The *rHLB* was calculated using the following formula [37]:

$$\sum_{i=1}^{n} w_i \times HLB_i \tag{1}$$

where w_i = the weight fraction of the emulsifier utilized, with its HLB_i.

Studies performed on formulations

Organoleptic assessments. In this study, properties such as appearance, consistence and color of the formulations were evaluated visually. Furthermore, the ability of the formulations to stretch on the skin, the absorption into the skin and odor has been estimated by sensorial analysis. For this purpose, ten female volunteers were tested the prepared emulsions in a laboratory with constant temperature, relative humidity and adequate light conditions. After applying the skin formulations on each half of their face, the volunteers have been estimated the sensory properties using a scale from 1 to 10.

pH measurement. Emulsions pH was measured using an inoLab pH meter.

Stability tests. All the obtained emulsions were subjected for 10 days to rapid stability tests, evaluating phase separation. For this purpose, the samples were kept at different temperatures: 25 ± 1 °C (at room temperature), 4 ± 0.1 °C (in refrigerator) and 40 ± 0.1 °C (in incubator). At the same time, the obtained formulations were kept for 6 months (at room temperature, in closed containers, protected from direct sunlight and humidity), to observe changes of visual aspect (homogeneity, color, fragrance, phase separation) and pH. A hot Incubator (Sanyo MIR-162, Japan) was used for incubation purpose.

Optical microscopy analysis was performed using a LEICA optical microscope model S8AP0, with Increase Power: 20–160x. The images were captured by an attached Nikon Coolpix MCD camera (Japan).

Rheological studies were conducted with a rotational viscometer Multi Visc-Rheometer (Fungilab, Spain) equipped with a TR 10 standard spindle and a Thermo Haake P5 Ultrathermostat to keep the sample at a constant temperature during the measurements. The operational conditions were detailed in a previous study [38]. The creams flow characteristics were determined at two temperatures: 24 °C (close to room temperature) and 33 °C (close to the human skin temperature). Briefly, for each sample the rotational speed varied from 0.3 to 60 rpm at both working temperatures, corresponding to a shear rate between 0.08 and 16.8 s^{-1}. The apparent viscosity was recorded versus shear rate and the corresponding rheological pattern was built. Also, for each formulation the forward and backward rheograms obtained by plotting shear stress versus shear rate were recorded.

Results and discussion

Formulation of oil/water emulsions

An emulsion is a mixture of two immiscible liquids with different polarities where one liquid is dispersed as spherical droplets into the other phase [39]. The emulsions used in cosmetic formulations are usually based on water (W)-oils mixtures (O). There are two main types of W–O systems: one of them consists of water droplets dispersed in an oil phase (called water-in-oil (W/O) emulsion); the other one is represented by dispersed droplets of oil in the aqueous phase (called oil-in-water (O/W) emulsion). As a general rule, the phase with higher concentration will tend to be the continuous phase. O/W emulsions are commonly used as water-washable drug bases and for general cosmetic purposes while W/O emulsions are widely used as emollients and for dry skin treatment [40].

The purpose of our study was to prepare and characterize some O/W skin cosmetic emulsions based especially on natural ingredients, most of them being obtained form vegetable materials. Thus, as oil phase, different mixtures of vegetable oils (avocado, jojoba, coconut and almond oils) were used. The vegetable oils, being rich in fatty acids and fatty alcohols provide the emollient properties while the presence of other constituents, such wax esters contributes to their occlusive properties [41]. Literature data also reported other dermatocosmetic properties of the mentioned vegetable oils. Thus, jojoba oil showed to be effective in enhancing the absorption of topical drugs while the high content of wax esters makes jojoba oil an active ingredient for the treatment of dermatoses with altered skin barriers, such as seborrheic dermatitis, eczematous dermatitis, atopic dermatitis, and acne. The topical application of avocado oil in experimental rats also showed an increase of collagen synthesis and a decrease of inflammatory cells number during the wound healing process [42]. Coconut oil also showed effectiveness in healing wounds by faster epithelization, also repairing the skin barrier and having antibacterial and antioxidant effect [43]. Almond oil is effective in ameliorating the itching of striae and preventing its progression [44]. As emulsifier, a plant based product containing glyceryl stearate and potassium stearate (recommended for O/W systems) was selected. Based on the quality certificate, Bella emulsifier is suitable for all skin types, especially for combination and oily skin; it can be used in a wide range of products including moisturizers, anti-wrinkle creams, eye creams, body milks, sun care, and color cosmetics formulations; the recommended dosage is 3–8 %; the addition of xanthan gum or a co-emulsifier contributes to the stabilization of fluid emulsions with low fat phase. Glyceryl stearate is reported to stabilize products, decrease water evaporation, make products freeze-resistant, and keep them from forming surface crusts [45]. The vegetable oils obtained form avocado, jojoba, coconut and almond were also used as fatty emollients, acting as lubricants on the skin's surface. Important to note that when applied topically, constituents of plant oils (triglycerides, phospholipids, free fatty acids, phenolic compounds and antioxidants) may act synergistically by several mechanisms: (1) promoting skin barrier homeostasis; (2) antioxidative activities; (3) anti-inflammatory properties; (4) anti-microbial properties; (5) promoting wound healing; and (6) anti-carcinogenic properties [46]. This is the reason for using in our study mixtures of vegetable oils to prepare the emulsion formulations.

The water phase was represented by lavender water as fragrant, calming and anti-bacterial ingredient, ideal for mild acne and for natural cleansing of the skin pores. Cosgard was added as antibacterial and antifungal preservative, avoiding the use of parabens. It is Ecocert approved for organic cosmetics, so it can also be used for sensitive skin and usually ensures preservation of cosmetic formulations containing water and flower water. The other ingredients of Cosgard mixture are: benzyl alcohol (fragrance, preservative, solvent, and viscosity-decreasing agent) [47]; salicylic acid (comedolytic agent, opens pores and encourages new skin to grow), glycerin (the most effective humectant available to increase stratum corneum hydration) [48]; ascorbic acid (stimulates collagen synthesis and is implied in antioxidant protection against UV-induced photodamage) [49].

It is important to note that preservatives are often raising issues due to their chemical structure, origin and/or allergic potential. In our study, besides Cosgard (an Ecocert approved preservative), a lemon and lavender essential oils (EOs) mixture (1:1) was also used as preservative (see Table 1). The EU Cosmetics Regulation settled the use of essential oils and single compounds in cosmetic products [50]. Eighteen of possible allergens can be found as ingredients of essential oils. The permissible concentration of these allergens must be lower than 0.001 % in body oils, massage oils and creams [51]. As reported in a previous paper, the EOs mixture used in the present study contains D-limonene (35.1 %) and linalool (22.4 %), considered by EU Cosmetic Regulation as possible allergens. Taking into account the concentration of each of the two essential oils in the prepared cosmetic emulsions (0.25 %), the concentrations of D-limonene (0.0009 %) and linalool (0.0006 %) in the formulations are below 0.001 %. As about Cosgard, it is accepted to use in natural cosmetics, typical recommended use level of Cosgard being up to 0.3–1 %. Depending on the type of formulation, it's best to use it at 1 %. We chose to replace half the amount of Cosgard with the essential oil mixture, taking into account the reported synergistic effect of synthetic preservatives with addition of essential oils in cosmetic preparations. The mixture of lavender and lemon essential oils (1:1 ratio) was also used as fragrance. The other constituents used to prepare the proposed formulations were: coconut powder, as natural exfoliant in facial and body scrubs; xanthan gum (an anionic polysaccharide with high-molecular-weight), used as binder, emulsion stabilizer, emulsifying surfactant, as well as an aqueous viscosity increasing agent in cosmetics (its high molecular weight being responsible for the high viscosity in solution); allantoin, made from urea and glyoxylic acid, which acts as skin-conditioning agent (miscellaneous and as skin protector) [52].

A preformulation study was conducted to obtain mixtures with different percentage content of individual vegetable oils (between 3 % and 20 %) and emulsifying agent (between 4 % and 6 %), the percent of oil phase varying between 21 % and 40 %. The formulations were prepared using oil mixtures with various ratios of vegetable oils, using the compositions presented in Table 1. Concerning the ingredients in solid phase, the content of collagen hydrolysate was maintained constant (1 %), while the other ingredients varied as follows: xanthan gum (0.2–1 %), allantoin (1–1.8 %) and coconut powder (1–1.3 %). The content of each preservative ingredient (Cosgard and mixture of essential oils) was kept constant in all formulation (0.5 %). After visual observation of ten formulations for 1 week, five formulations were selected, that show stability, no phase separation, sedimentation, creaming or flocculation.

Organoleptic assessments

The obtained emulsions present different color according to the vegetable oil content (Fig. 1): emulsion no. 1 is yellow in color due to the high content in avocado oil, no. 2, 4 and 5 have a slightly yellowish color (lower content of avocado oil), and no. 3 has a white color, this one being prepared without adding avocado oil.

Fig. 1: O/W emulsions.

All emulsions have a pleasant, creamy, moisturized appearance and the fragrance specific to the essential oils in the composition. The results of the sensory analysis presented in Fig. 2 show that all cosmetic formulations are acceptable by consumers. The emulsions can be gently stretched, being easily absorbed into the skin and having a pleasant odor. Moreover, the prepared emulsions do not irritate or leave residue on the skin.

pH measurement

The pH values of the obtained emulsions are shown in Table 2.

The natural pH of the skin varies between 4.5 and 6.5 due to race, skin tone or color, exposure to sunlight and diseases. This interval is considered to be optimal for cosmetic products that come into direct contact with the skin; also, the products administered on the skin can change skin pH. Skin pH is one of the most important biophysical features following sebum [53]. The values obtained for the emulsions are in this interval, indicating that emulsions can be safely applied on the skin.

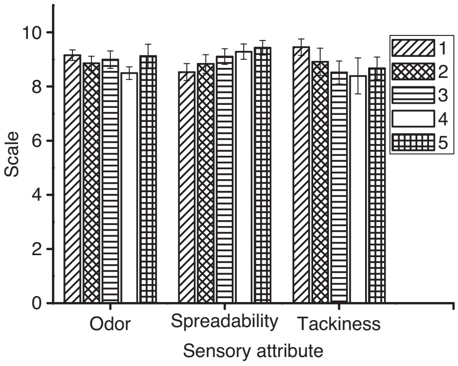

Fig. 2: Sensory evaluation of the emulsion formulations no. 1–5.

Table 2: The pH values for the obtained emulsions.[a]

	1	2	3	4	5
pH of fresh sample	5.56 ± 0.27	5.67 ± 0.22	5.75 ± 0.34	5.78 ± 0.28	5.61 ± 0.33
pH of sample after 6 months	5.60 ± 0.28	5.68 ± 0.22	5.78 ± 0.35	5.81 ± 0.29	5.65 ± 0.34

[a] The results represent the mean of three independent measurements ± standard deviation.

Stability tests

Different variables are responsible for the stability of emulsions, such as: the composition of oils, the kind of emulsifier and the hydrophilic-lipophilic balance (*HLB*) value of blend of emulsifiers. Emulsions prepared with unsaturated emulsifiers and highly unsaturated vegetable oils (containing mainly oleic and linoleic acids) were found to be more stable than those prepared with some unsaturated emulsifiers and vegetable oils consisting of less unsaturated fatty acids [54]. The approximate chain length distribution and degree of unsaturation of the vegetable oils used in our study for preparing the emulsifiers are presented in Table 3.

As presented in Table 3, almond and avocado oils contain higher percents of monounsaturated long chain fatty acids, (especially oleic (C18:1) and linolenic (C18:1) acids), followed by jojoba oil which is rich in erucic acid (C22:1). The coconut has a low content of unsaturated fatty acids, being rich in saturated fatty acids (~90 %), with a high content (50 %) of lauric acid (with relatively short chain, C12:0). These compounds could influence the *HLB* value of emulsifier blends. *HLB* is considered to be a measure of stability of an emulsion. The "*required HLB*" or "*effective HLB*" value is the *HLB* of the surfactant, or mixture of surfactants, which allows the most stable emulsion for a given system (processing conditions, oil phase, etc.) to be obtained [56]. The *rHLB* value depends on the chain length(s) of the fatty acids attached to the emulsifier; short fatty acid chains leading to higher *rHLB* values [57]. This assumption is confirmed by the *rHLB* values of the vegetable oils used in our study. Thus, almond oil, with high content of unsaturated C18 fatty acids has the lower *rHLB* value (6), while coconut oil, with the lower content of C18 unsaturated fatty acids and a high content of saturated fatty acids (C12) has the highest *rHLB* value (8). In practice, the best emulsification for most systems is provided by mixed emulsifiers, and most O/W cosmetic emulsions have *rHLB*s ranging from about 6 to 15 [58].

The *rHLB* values of the individual components used to prepare the oil phases are as follows [59]: almond oil, 6; avocado oil, 7; coconut oil, 8; jojoba oil, 6–7; Bella emulsifier, 5.8. The *rHLB* values of the obtained emulsifier blends (calculated using the equation (1)) are presented in Table 4. In our study, the proposed emulsifier blends are characterized by *rHLB* values between 6.00 and 7.34, confirming the stability of the obtained O/W emulsions. As we observe, the emulsifier blend no. 3 does not contain jojoba and avocado oils, being characterized by the lowest value of *rHLB*. This could be explained by the low content of unsaturated

Table 3: An approximate chain length distribution and the degree of unsaturation of the vegetable oils used for preparation of emulsions [55].

Oil	Unsaturated fatty acids (%)				
	Monounsaturated				Biunsaturated
	Palmitoleic acid (C16:1)	Oleic acid (C18:1)	Eicosenic acid (C20:1)	Erucic acid (C22:1)	Linoleic acid (C18:2)
Almond		60–86			7–30
Avocado	5–80	60–80			10–20
Coconut		4–12			2
Jojoba		5–15	65–80	10–20	

Table 4: *rHLB* values of the obtained emulsifier blends.

Emulsifier blend, no.	Ratio, w/w COC:ALM:JOJ:AVC:B[a]	*rHLB* of the emulsifier blend
1	10:10:10:10:5	6.87
2	10:10:5:5:5	7.34
3	20:10:0:0:5	6.00
4	20:10:5:5:5	6.44
5	10:5:3:3:4	6.08

[a] COC, Coconut oil; ALM, almond oil; JOJ, jojoba oil; AVC, avocado oil; B, Bella emulsifier.

fatty acids. The emulsifier no. 2 (based on coconut and almond oils) is characterized by the highest *rHLB* value. This result is in agreement with literature, on the basis of which, a blend of high and low *rHLB* surfactants is often used to achieve the desired value in part because of demonstrated effectiveness and efficiencies in packing at the interface [60].

The stability tests showed that the samples containing 4 % and 5 % emulsifier were stable at all three working temperatures. It was observed that the emulsifier concentration higher than 5 % destabilized the emulsions, this behavior being in line with literature data [61].The physical characteristics of the obtained emulsions (pH, color, phase separation, liquefaction) were also evaluated 6 months after preparation (the samples were stored at room temperature, in closed containers). The prepared formulations were found to be stable in structure, without any change in physical appearance and were found to keep the uniform appearance as in the first day. Any phase separation was not observed in formulations assessed visually. All samples proved to be stable over the period, their physical characteristics being unchanged. Actually, no significant change of aspect, odor and pH was observed. For example, pH pair values of the five samples (measured before and after 6 months) were found to be very close (Table 2).

Optical microscopy analysis

Results of the optical microscopy analysis presented in Fig. 3 show that all emulsions have foam-like appearance (the oil droplets are separated by a thin film of continuous phase) and are slightly aerated (it was obtained structures capable of retaining bubble integrity). As it can be seen in Fig. 3, there are more uniform (a, d, e) or less uniform (b, c) dispersed oil droplets; also, the particle size differs from one sample to another, the content of different vegetable oils being responsible for these differences because of their different content in fatty acids (oleic acid, linoleic acid, palmitic acid, etc.). The foam-like structure of the samples created during shear blending confer them a viscoelastic rheological behavior, responsible for their appearance [62].

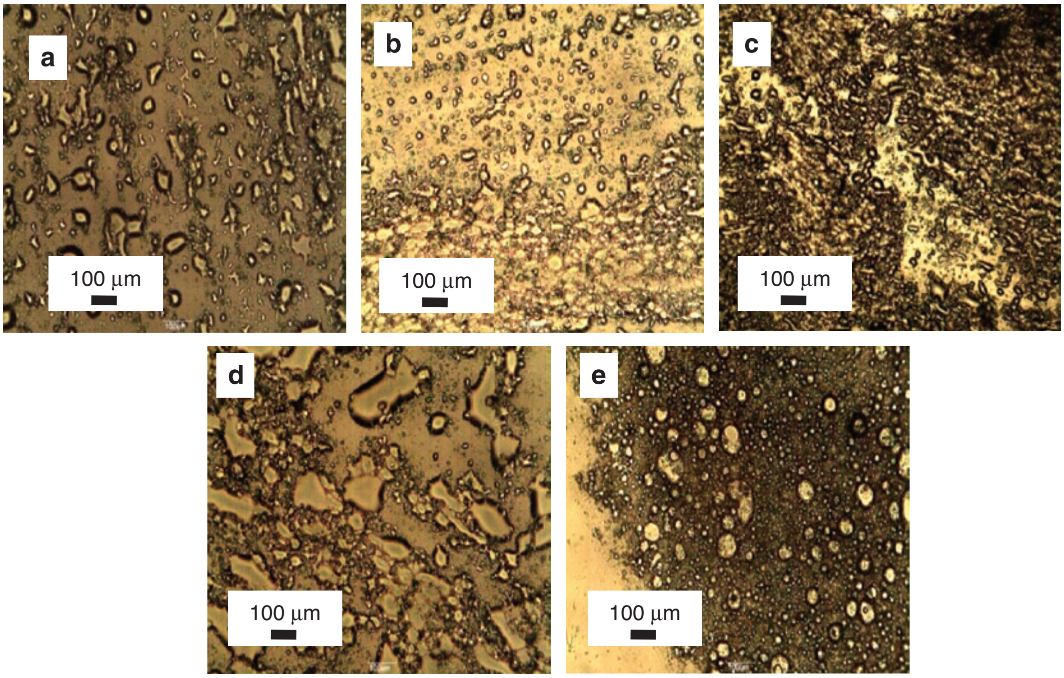

Fig. 3: Images obtained by optical microscopy for emulsion variants 1–5 (a–e), power × 100.

Rheological studies

The main rheological parameters estimated in the quality control of the cosmetic emulsion are shear (apparent) viscosity (η) and thixotropy.

Viscosity

The apparent viscosity is defined as the ratio between shear stress (σ) and shear rate ($\dot{\gamma}$):

$$\eta = \sigma / \dot{\gamma} \tag{2}$$

The pseudoplastic fluids, such as cosmetic emulsion formulations are characterized by flow curves in which viscosity decreases while shear rate increases [63]. The apparent viscosity is related to different formulation properties depending on the shear rate range. A flow curve, viscosity (η) versus shear rate ($\dot{\gamma}$), across a wide range of shear rates, can provide important information about storage stability. The rheological patterns apparent viscosity versus shear rate were obtained for the formulations analyzed at 24 °C and 33 °C and are presented in Figs. 4 and 5.

As shown in Figs. 4 and 5, the most viscous is the system no. 1 (containing 5 % glyceryl stearate-potassium stearate based emulsifier and 1 % xanthan gum), while the system no. 5 (containing 4 % emulsifying agent and 0.2 % xanthan gum) exhibits the lowest viscosity. By decreasing the concentration of xanthan gum from

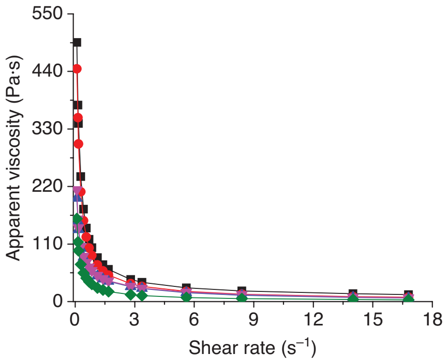

Fig. 4: Rheological diagrams: apparent viscosity versus shear rate for emulsions analyzed at 24 °C. Emulsions no.: (1) ■; (2) ●; (3) ▲; (4) ▼; (5) ◆.

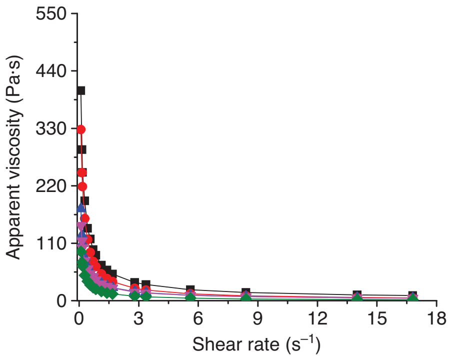

Fig. 5: Rheological diagrams: apparent viscosity versus shear rate for emulsions analyzed at 33 °C. Emulsions no.: (1) ■; (2) ●; (3) ▲; (4) ▼; (5) ◆.

1 % to 0.2 %, a decrease of viscosity was observed (see the viscosity profiles of samples no. 1–5). The samples no. 3 and 4 have similar viscosity profiles, in good agreement with their identical content of glyceryl stearate-potassium stearate based emulsifier (5 %) and xanthan gum (0.2 %). Also, it was observed that for the same level of xanthan gum concentration, samples no. 4 and 5 have different viscosities, the sample no. 5 being characterized by the smallest viscosity. This behavior is attributed to the emulsifier content (5 % for sample no. 4 and 4 % for sample no. 5).

The viscosity variation for the obtained emulsions could be attributed to the variation of their ingredients, especially to the concentration of the emulsifier and the rheology modifier. Xanthan gum is the rheology modifier usually added into the emulsion formulations. An emulsion formulation requires a rheology modifier which has the role to control the consistency of the final product. Without xanthan gum, the formulation systems were reported to be very fluid, the emulsification process being not affected by the viscosity level [64]. The presence of xanthan gum increases the resistance of emulsions to flow, increasing the viscosity; this behavior is explained by the fact that xanthan gum reduces the interfacial tension and tends to form a cohesive interfacial film around the emulsion droplets, improving the stability of the systems [65]. Also, it was assumed that xanthan gum has the property to keep away the droplets one from each other by forming steric stabilizing polymer-coated particles as it has sufficient polymer chain to cover the surface of oil droplets thus maintaining their sizes [66]. Concerning the glyceryl stearate-potassium stearate based emulsifier, it exhibits two favorable inputs: an interfacial tension decrease between the two immiscible liquids and the stabilization of the dispersed phase against coalescence [67]. Based on the rheological results, we assume that the mixtures containing 4 % and 5 % emulsifier, ensured homogeneity in coating of the interface of oil/water droplets. The rheological profiles presented in Figs. 4 and 5 also show the influence of temperature on the viscosity of the tested products, which decreases with increasing temperature. For all emulsions, the viscosity of the tested systems decreases with increasing shear rate at both temperatures, indicating a pseudoplastic non-Newtonian behaviour with shear thinning. The pseudoplastic flow behavior is adequate for a cosmetic emulsion because the decrease of its apparent viscosity with increasing stress makes the system more fluid, thus, facilitating the flow [68].

To quantify the pseudoplastic behaviour of the obtained products, the Power law viscosity model was used. It expresses the relationship between apparent viscosity (η, expressed in Pa·s). and shear rate ($\dot{\gamma}$, expressed in s^{-1}) of a fluid exhibiting non-Newtonian behavior and it is described by equation (3):

$$\eta = m \times \dot{\gamma}^{n-1} \tag{3}$$

where m and n are the parameters evaluated through the linearization of equation (3) by double logarithmic method [69]. The m parameter is the consistency coefficient (expressed in Pa·s^n) and its value is correlated with the viscosity obtained for the shear rate value of 1·s^{-1}; the n parameter is the flow behavior index (called power-law-index, dimensionless) [69].

The values of the rheological parameters (m and n), characteristic for this model, as well as the statistical parameter (R-squared coefficient, R^2), are presented in Table 5, for the formulations tested at 24 °C and 33 °C.

The results in Table 5 show that the determination coefficient R^2 values are between 0.9919 and 0.9986 for all tested emulsions at both temperatures. This behavior indicates that the Power law model fitted well the rheological experimental data. The rheological parameter m is numerically equal to viscosity at 1 s^{-1}, being a measure of the consistency of the emulsion. Thus, at a constant working temperature (24 °C and 33 °C, respectively), the value of m decreases from sample no. 1 to sample no. 5, in agreement with the viscosity profiles illustrated in Figs. 4 and 5 (the highest m value being associated with the highest viscosity of sample no. 1, while the lowest m value is associated with the lowest viscosity of sample no. 5). Also, the parameter m is strongly influenced by the temperature, decreasing about 1.27–1.47 times with increasing temperature. The power-law-index, n, which indicates how shear thinning, varies between 0.54 and 0.67, denoting pseudoplastic or shear-thinning behavior [69] of the obtained cosmetic formulations. For a pseudoplastic fluid, n is less than 1 [70]. As observed in Table 5, the values of the flow behavior index, n, are slightly affected by the temperature which is in agreement with the literature data [71].

Table 5: The values of the rheological parameters *m* and *n* and of the coefficient R^2, specific to Power law model applied to the emulsions analyzed at 24 °C and 33 °C.

Emulsion	Consistency coefficient, *m* (Pa·s^n)		Flow behavior index (dimensionless), *n*		Determination coefficient, R^2	
	24 °C	33 °C	24 °C	33 °C	24 °C	33 °C
1	97.417	77.202	0.660	0.657	0.9953	0.9973
2	83.759	61.108	0.682	0.681	0.9919	0.9941
3	51.310	36.133	0.541	0.627	0.9975	0.9984
4	52.618	35.687	0.555	0.568	0.9986	0.9944
5	29.444	20.428	0.670	0.624	0.9982	0.9928

Thixotropy

Another important parameter used for the assessment of this kind of skin cosmetic products is the thixotropy [72]. Reflecting a non-Newtonian behavior, thixotropy is a very important property of the plastic and pseudoplastic flow dispersion systems which show a time-dependent change in the viscosity [73]. Thixotropic behavior describes a degradation of a sample during a constant shear loading phase (a decrease in viscosity with time, when shear is applied at a constant value); during the relieved phase, the original structure is recoverable. The thixotropic behavior of the obtained emulsions is graphically illustrated in Fig. 6a–e through the forward and backward rheograms, recorded by plotting shear stress versus shear rate, at both temperatures.

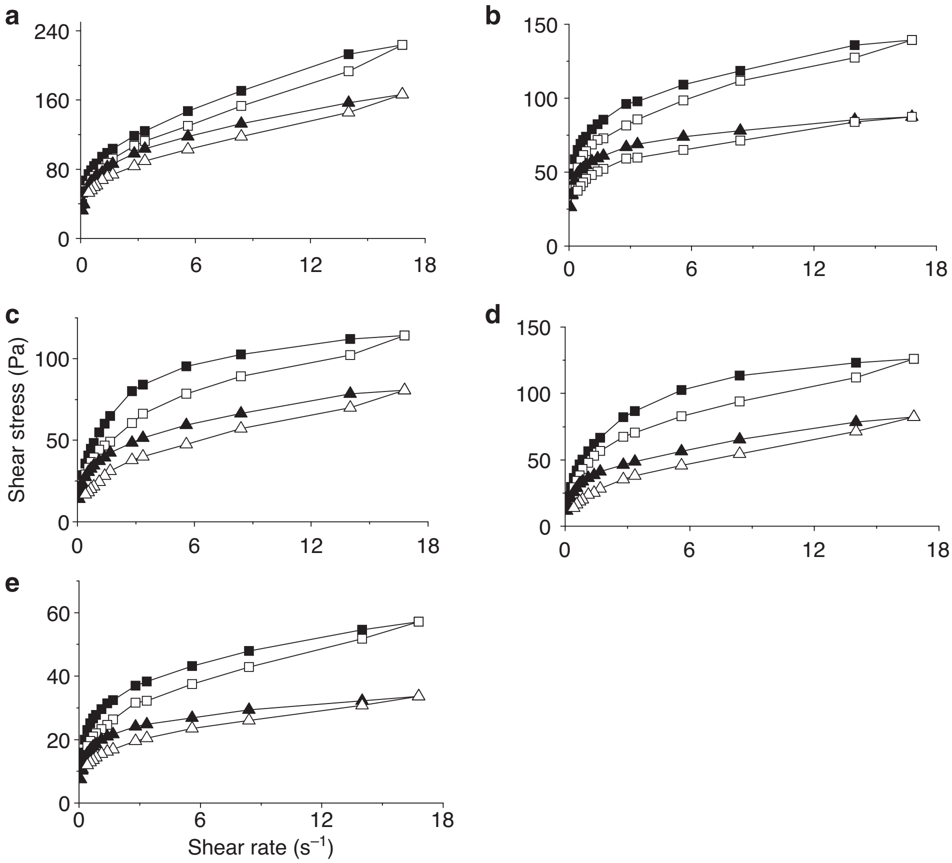

Fig. 6: (a–e) Forward and backward rheograms for the emulsion no. 1–5, at 24 °C (forward (■) and backward rheograms (□)) and at 33 °C (forward (●) and backward rheograms (○)).

Table 6: The values of thixotropy parameters for the emulsions tested at 24 °C and 33 °C.

Emulsion	S_{fwd} (Pa·s⁻¹)		S_{bw} (Pa·s⁻¹)		S_{thix} (Pa·s⁻¹)		T_{hyst} (%)	
	24 °C	33 °C	24 °C	33 °C	24 °C	33 °C	24 °C	33 °C
1	2760.693	2131.718	2492.229	1916.323	268.464	215.395	9.72	10.10
2	1919.199	1266.830	1760.387	1160.094	158.812	106.736	8.27	8.42
3	1596.710	1059.422	1384.850	902.116	211.860	157.306	13.26	14.84
4	1733.345	1043.205	1495.488	886.657	237.857	156.548	13.72	15.01
5	764.486	469.254	687.188	418.500	77.298	50.754	10.11	10.82

The rheograms in Fig. 6a–e indicate a thixotropic behavior for all emulsions analyzed, at both temperatures, the presence of a hysteresis area meaning that a sample shows time dependence rheological behavior (thixotropy). The thixotropic character was quantified by two specific parameters [74]:

a) thixotropy area (S_{thix}) – the area enclosed between forward (S_{fwd}) and backward (S_{bw}) curves;
b) thixotropy index (T_{hyst}%) – evaluated according to eq. (4):

$$T_{hyst},\ \% = \left(\frac{S_{fwd} - S_{bw}}{S_{fwd}}\right) \cdot 100 \qquad (4)$$

Due to experimental errors occurred in the rheological measurements, only the T_{hyst}% values higher than 5 % are significant.

The thixotropic parameters values are listed in Table 6 for the emulsions tested at 24 °C and 33 °C.

The thixotropic index values from Table 6, higher than 5 %, show thixotropic behavior for all emulsions, at both temperatures. Also, at the same working temperature, the hysteresis area values (S_{thix}) decrease form the most viscous sample (no. 1) to the least viscous sample (no. 5). This rheological parameter represents an indicator for the degree of system destructuration, higher values for thixotropic area indicating a higher thixotropy [74]. The S_{fwd}, S_{bw} and S_{thix} are influenced by the working temperature. The thixotropic parameters decrease about 1.3–1.7 times with increasing temperature. High values for thixotropic area and respectively for thixotropic index are associated with the transformation of an initially viscous emulsion in a thin product with a high degree of spreadability and formation of a continuous film on the skin. Moreover, thixotropy is a property sought for the semisolid dosage forms as it ensures a long retention time enhancing the efficacy of the formulation at the administration site.

Conclusions

In this study, instead of common compounds used in cosmetic preparations, which shows allergies risk, we prepared some O/W emulsion formulations using hydrolysate collagen (a valuable protein in leather industry with antimicrobial properties), mixtures of vegetable oils, essential oils and other natural ingredients. The pH values of the obtained formulations were found to be in accordance with the human skin pH. Also, they present the two quality parameters usually estimated for topical cosmetic emulsions, namely pseudoplastic behavior (quantified by applying the rheological model of the Power law – viscosity depending on the shear rate) and thixotropic behavior (quantified by thixotropic area and thixotropy index). The tested cosmetic formulations showed a non-Newtonian pseudoplastic flow with shear thinning at both selected temperatures (24 °C and 33 °C). The flow parameters and thixotropy characteristics determined were markedly influenced by both the composition of the cosmetic formulations (especially by the concentration of the emulsifier based on glyceryl stearate and potassium stearate) and of the rheology modifier, namely xanthan gum) and the working temperature. The cosmetic formulations proved the initial characteristics (appearance, consistence, color, odor and pH) for 6 months (at room temperature). No phase separation was observed, the formulations being stable in the period of time mentioned. Supplementary studies are on-going, aiming to obtain dermatocosmetic products based on the emulsion formulations reported in this paper.

References

[1] T. Mitsui. In *New Cosmetic Science* (1st ed.), pp. 121–147, Elsevier, Amsterdam, The Netherlands (1997).
[2] L. Chularojanamontri, P. Tuchinda, K. Kulthanan, K. Pongparit. *J. Clin. Aesthet. Dermatol.* **7**, 36 (2014).
[3] J. Q. Del Rosso, M. Alam. *Cosmeceuticals* (2nd ed.), pp. 97–102, Saunders Elsevier, China (2009).
[4] M. Lodén. *J. Eur. Acad. Dermatol. Venereol.* **19**, 672 (2005).
[5] H. De Wet, S. Nciki, S. F. Van Vuuren. *J. Ethnobiol. Ethnomed.* **9**, 1 (2013).
[6] H. C. Williams, R. P. Dellavalle, S. Garner. *Lancet* **379**, 361 (2012).
[7] H. Muda, A. Aziz, Z. Mat Taher, R. A. Aziz. In *Recent Trends in Malaysia Medicinal Plants Research* (First ed.), pp. 126–175, UTM Press, Bahru Johor, Malaysia (2017).
[8] B. Fonseca-Santos, M. Antonio Corrêa, M. Chorilli. *Braz. J. Pharm. Sci.* **51**, 17 (2015).
[9] D. Chanchal, S. Swarnlata. *J. Cosmet. Dermatol.* **7**, 89 (2008).
[10] L. Bajerski, L. R. Michels, L. M. Colomé, E. A. Bender, R. J. Freddo, F. Bruxel, S. E. Haas. *Braz. J. Pharm. Sci.* **52**, 347 (2016).
[11] E. Yara-Varón, Y. Li, M. Balcells, R. Canela-Garayoa, A. S. Fabiano-Tixier, F. Chemat. *Molecules* **22**, 1474 (2017).
[12] A. V. Bailey, R. A. Pittman. *J. Am. Oil Chem. Sot.* **4B**, 775 (1971).
[13] J. N. Hadi, M. H Norazian, A. Kausar. In *Current Issues in Pharmacy*, Q. U. Ahmed (Ed.), pp. 178–195, IIUM Press, Kuala Lumpur (2011).
[14] J. Pavlačková, K. Kovacsová, P. Radiměřský, P. Egner, J. Sedlaříková, P. Mokrejš. *Braz. J. Pharm. Sci.* **54**, 1 (2018).
[15] A. M. R. Alvarez, M. L. G. Rodriguez. *Grasas y Aceites* **51**, 74 (2000).
[16] S. S. The, S. H. Mah. *J. Oleo Sci.* **67**, 138 (2018).
[17] S. S. Gani, S. Mustafa, S. Arifin, N. F. Mokhtar, U. H. Zaidan, Proceedings of ISER 108th International Conference, Mecca, Saudi Arabia, 23rd to 24th February (2018).
[18] A. R. Rahate, J. M. Nagarkar. *J. Dispers. Sci. Technol.* **28**, 1077 (2007).
[19] M. Kowalska, M. Ziomek, A. Żbikowska. *Int. J. Cosmet. Sci.* **37**, 408 (2015).
[20] K. Oluyemisi, F. Egharevba, H. Omoregie, A. P. Ochogu. *Int. J. Biodivers. Conserv.* **4**, 101 (2012).
[21] W. V. Magalhães, A. R. Baby, M. V. R. Velasco, D. M. M. Pereira, T. M. Kaneko. *Braz. J. Pharm. Sci.* **47**, 693 (2011).
[22] https://www.premiumbeautynews.com/en/organic-monitor-gives-predictions, 10997.
[23] E. Dănilă, Z. Moldovan, M. Popa, M. C. Chifiriuc, A. D. Kaya, M. Albu Kaya. *Ind. Crops Prod.* **122**, 483 (2018).
[24] M. Dreger, K. Wielgus. *Herba Pol.* **59**, 142 (2013).
[25] A. M. Maccioni, C. Anchisi, A. Sanna, C. Sardu, S. Dessi. *Int. J. Cosmet. Sci.* **24**, 53 (2002).
[26] A. Yorgancioglu, E. E. Bayramoglu. *Ind. Crops Prod.* **44**, 378 (2013).
[27] M. I. Avila Rodriguez, L. G. Rodriguez Barroso, M. L. Sanchez. *J. Cosmet. Dermatol.* **17**, 20 (2018).
[28] S. Sibilla, M. Godfrey, S. Brewer, A. Budh-Raja, L. Genovese. *Open Neutraceutical J.* **8**, 29, (2015).
[29] J. Fan. *Nutrients* **5**, 223 (2013).
[30] T. Silva, J. Moreira-Silva, A. Marques, A. Domingues, A. Bayon, R. Reis. *Mar. Drugs* **12**, 5881 (2014).
[31] N. A. Binti Abd Samad, A. Singh Sikarwar. *Int. J. Biochem. Res. Rev.* **14**, 1 (2016).
[32] E. Jeevithan, Z. Qingbo, B. Bao, W. Wu. *J. Nutr. Ther.* **2**, 218 (2013).
[33] Y. Y. Peng, V. Glattauer, J. A. Werkmeister, J. A. M. Ramshaw. *Int. J. Cosmet. Sci.* **55**, 327 (2004).
[34] V. Trandafir, G. Popescu, M. G. Albu, H. Iovu, M. Georgescu, *Collagen Extraction Techniques* (in Roumanian), Chapter 4, pp. 111–112, Ars Docendi, Bucharest (2007).
[35] Regulation (EC) no 1223/2009 of the European Parliament and of the Council of 30 November 2009 on Cosmetic Products, p. 59 (2009).
[36] A. Yorgancioglu, E. Eke Bayramoglu. *Ind. Crops Prod.* **44**, 378 (2013).
[37] W.C. Griffin. *J. Soc. Cosmet. Chem.* **1**, 311 (1949).
[38] M. V. Ghica, M. G. Albu, C. Dinu-Pîrvu, Ș. Moisescu. *Rev. Chim.* **63**, 929 (2012).
[39] Y. Zhao, N. Khalid, G. Shu, M. A. Neves, I. Kobayashi, M. Nakajima. *Int. J. Food Prop.* **20**, 1329 (2017).
[40] S. Smaoui, H. Ben Hlima, R. Jarraya, N. Grati Kamoun, R. Ellouze, M. Damak. *Afr. J. Biotechnol.* **11**, 9664 (2012).
[41] A. Sethi, T. Kaur, S. Malhotra, M. Gambhir. *Indian J. Dermatol.* **61**, 279 (2016).
[42] A. P. de Oliveira, S. Franco Ede, R. Rodrigues Barreto, D. P. Cordeiro, R. G. de Melo, C. M. de Aquino, A. A. E Silva, P. L. de Medeiros, T. G. da Silva, A. J. Goes. *Evid. Based Complement. Alternat. Med.* **2013**, Article ID 472382, 8 pages (2013).
[43] K. G. Nevin, T. Rajamohan. *Skin Pharmacol. Physiol.* **23**, 290 (2010).
[44] M. Hajhashemi, M. Rafieian, H. A. Rouhi Boroujeni, S. Miraj, S. Memarian, A. Keivani, F. Haghollahi. *J. Matern. Fetal Neonatal Med.* **31**, 1703 (2018).

[45] https://www.cosmeticsinfo.org/products/moisturizers.

[46] T.-K. Lin, L. Zhong, J. L. Santiago. *Int. J. Mol. Sci*. **19**, 70 (2018).

[47] B. Nair. *Int. J. Toxicol.* **20**, 23 (2001).

[48] Z. D. Draelos, L. A. Thaman. *Cosmeceuticals* (2nd ed.), pp. 173–174, Saunders Elsevier, China (2009).

[49] J. M. Pullar, A. C. Carr, M. C. M. Vissers. *Nutrients* **9**, 1 (2017).

[50] European Directorate for the Quality of Medicines & Health Care of the Council of Europe. Guidance on Essential Oils in Cosmetic Products. Available online: https://www.edqm.eu/en/guidance-essential-oils-cosmetic-products.

[51] The European Communities. Labelling of ingredients in cosmetics Directive 76/768/EEC. Available online: http://ec.europa.eu/consumers/sectors/cosmetics/files/doc/guide_labelling200802_en.pdf.

[52] https://www.cosmeticsinfo.org/products/moisturizers.

[53] S. Smaoui, H. B. Hlima, R. Jarraya, N. Grati Kamoun, R. Ellouze, M. Damak. *Afr. J. Biotechnol.* **11**, 9664 (2012).

[54] N. Garti, G. F. Remon. *J. Food Technol*. **19**, 711 (1984).

[55] A. Zielinska, I. Now, A. K. *Chemik* **68**, 103 (2014).

[56] C. Genot, T.-H. Kabri, A. Meynier. "Chapter 5 – Stabilization of omega-3 oils and enriched foods using emulsifiers", in *Food Science, Technology and Nutrition*, p. 163, Woodhead Publishing Limited, Cambridge, UK (2013).

[57] N. Anarjan, C. P. Tan. *Molecules* **18**, 768 (2013).

[58] IFSCC Monograph No. 4, *Introduction to Cosmetic Emulsions and Emulsification*, p. 16, Published by Micelle Press, 12 Ullswater Crescent, Weymouth, Dorset DT3 5HE, England (1997).

[59] J. N. Hadi, M. H. Norazian, A. Kausar. In *Current Issues in Pharmacy*, Q. U. Ahmed (Ed.), pp. 178–195, IIUM Press, Kuala Lumpur (2011).

[60] Convergent Cosmetics. Emulsions and the HLB System. Available online: http://www.lotioncrafter.com/pdf/Emulsions_&_HLB_System.pdf.

[61] C. D. Vaughan, D. A. Rice. *J. Dispers. Sci. Technol*. **11**, 83 (1990).

[62] A. R. Rahate, J. M. Nagarkar. *J. Dispers. Sci. Technol.* **28**, 1077 (2007).

[63] C. Solans, R. Pons, H. Kunieda. In *Modern Aspects of Emulsion Science, The Royal Society of Chemistry*, B. P. Binks (Ed.), pp. 367–394, Cambridge, UK (1998).

[64] T. Tadros. *Adv. Colloid Interface Sci.* **227**, 108 (2004).

[65] S. H. Saharudin, Z. Ahmad, M. Basri. *Int. Food Res. J.* **23**, 1361 (2016).

[66] N. Akhtar, B. Khan, M. Khan, T. Mahmood, H. Khan, M. Iqbal, S. Bashir. *Int. J. Pharmacol. Pharm. Sci*. **5**, 128 (2011).

[67] V. Kumar, L. Wang, M. Riebe, H. Tung, R. K. Prud'homme. *Mol. Pharm.* **6**, 1118 (2009).

[68] T. Moravkova, P. Filip. *Adv. Mater. Sci. Eng*. **2013**, Article ID 168503, 7 pages (2013).

[69] P. S. Prestes, R. B. Rigon, N. M. N. Corrêa, G. R. Leonardi. *Rev. Ciênc. Farm. Básica Apl.* **30**, 37 (2009).

[70] L. M. Julio, V. Y. Ixtaina, M. Fernandez, R. M. T. Sanchez, S. M. Nolasco, M. C. Tomas. *J. Food Sci. Technol.* **53**, 3206 (2016).

[71] L. L. Schramm. *Emulsions, Foams, and Suspensions: Fundamental and Applications*, pp. 174. Wiley-VCH, Weinheim, Great Britain (2010).

[72] M. K. Krokida, Z. B. Maroulis, G. D. Saravacos. *Int. J. Food Prop.* **4**, 179 (2001).

[73] L. R. Gaspar, P. M. Campos. *Int. J. Pharm.* **250**, 35 (2003).

[74] C. H. Lee, V. Moturi, Y. Lee. *J. Control. Release* **136**, 88 (2009).