# OBTAINING AND CHARACTERIZATION OF SOME EMULSIONS BASED ON COLLAGEN HYDROLYSATE AND NATURAL EXTRACTS WITH A POTENTIAL ANTICELLULITIC ACTION

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*The aim of this study was to formulate and evaluate O/W emulsions based on collagen and naturals ingredients such as vegetable oils and butters, caffeine, ivy extract and pink pepper essential oil. The essential oil of pink pepper was obtained by hydrodistilation and characterized by GC-MS. The main components of this essential oil are: α-Phelandrene 35.84%, Limonene 17.31%, α-Pinene 1.98% (monoterpenes) and β-Phelandrene 13.04%, o-Cymene 4.65%, δ-Cadinene 3.27%, trans-Caryophyllene3.04%, Bicyclogermacrene 2.13% (sesquiterpenes). The emulsions obtained are stable and the pH values of emulsions correspond to the natural pH of the skin, indicating that emulsions can be safely applied to the skin. Results from the optical microscopy analysis show that emulsions have a creamy and foam "like" appearance. All the emulsions present adequate rheological properties. Further microbiological tests and other analysis are necessary for the obtained emulsions.*

**Keywords**: collagen, essential oils, natural extracts, cellulite.

## 1. Introduction

Cellulite is a clinical and aesthetic condition affecting most women. It may appear in preadolescence, adolescence, or adulthood. Cellulite is a very common topographical alteration in which the skin acquires an orange peel or mattress appearance [1]. With cellulite, alterations occur to the adipose and connective tissue, resulting a blood and lymphatic alterations [2]. Several authors have classified cellulite into four clinical stages or degrees, from 0 to III, depending on the alteration to the skin surface.

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Cellulite is a complex pathology that involves the epidermis, dermis, and subcutaneous tissue. Several theories of cellulite pathophysiology have been formulated during recent years. These theories can be subdivided into three groups: (i) an increase in water content as the concentration of proteoglycans rises, leading to the development of an edema; (ii) a change in the local microcirculation; (iii) an abnormal arrangement of collagen structures in the tissue [3].

Type I collagen was reported as a major target in cellulite. One of the theories on the etiology of cellulite is based on the collagen breakdown in the dermis. Some studies relate that estrogen provokes alterations in collagen. In fact, cellulite worsens with pregnancy, menstrual cycle, use of contraceptives, and hormonal replacement [4]].

A series of treatments have been used over the time to treat or improve the appearance of cellulite, such as topical products (cosmetics), oral supplements, devices (mechanical massage, lasers, light sources, radiofrequency, and other technologies), and surgical procedures (liposuction) [5].

Topical anticellulite preparations may be divided in 4 major groups according to their mechanism of action. These treatments include agents that increase the microcirculation flow (Ginkgo biloba, *Centellaasiatica*, Papaya, Pineapple, Red grapes, Ivy extracts), reduce lipogenesis and promote lipolysis (caffeine), restore the normal structure of dermis and subcutaneous tissue (retinoic acid and vitamin A derivatives which increase the collagen synthesis), and prevent free radical formation or scavenge free radicals (ascorbic acid and vitamin E). Common ivy (*Hedera helix*) leaves have flavonoids, such as rutosid and rutinosid, and saponins, such as hederin, hederacosid, and hederagenin. Their fruits have saponins, especially hederin, and the trunk has gomoresins and saponins. All saponins improve venous and lymphatic drainage and reduce edema. The compound hederin also has an analgesic and anti-inflammatory effect, vasoconstrictor and antiexudativeproperties, and can also reduce capillary permeability. It activates the circulation which aids drainage of the infiltrated tissue and reduces inflammation. It is used for cellulite treatment in concentrations of 2%. Caffeine penetrates the skin very easily, which facilitates its absorption and action. Caffeine acts directly on adipose cells, promoting lipolysis and inhibiting phosphodiesterase. Caffeine also has a stimulating effect on the cutaneous microcirculation [6].

In the last years the interest in the pharmaceutical and cosmetic industry to use plant extracts for various fields of application was substantially growing, due to the adverse effects generated by synthetic ingredients [7]. Essential oils have been used to improve the health and physical appearance of the human body, and to protect the skin against the environment damage since ancient times [8]. Essential oils are complex mixtures containing dozens of substances of various

chemical composition at different concentrations [9]. They are characterized by the compounds present in highest concentration which determine their flavour, fragrance and biological properties [10]. Biological proprieties of essential oils such as antibacterial, antifungal and antioxidant activity make essential oils suitable for use in cosmetics [11,12]. A previous study showed that essential oilscan be used as a natural alternative for some skin disease, reducing the side effect of the conventional treatments [13]. Regarding cellulite, some essential oils have been reported to be effective in treating this pathology: grapefruit (*Citrus paradisi* L.), cedarwood (*Cedrusatlantica*Manetti), lemongrass (*Cymbopogon citratus* L.), juniper (*Juniperusvirginiana*L.), geranium (*Pelargonium odoratissimum* L.), rosemary (*Rosmarinusofficinalis* L.), lavender (*Lavandula angustifolia* L.), andarin (*Citrus reticulata*L.), lemon (*Citrus limon* L.), black pepper (*Piper nigrum* L.) and pink pepper (*SchinusMolle*L.) [14].

The aim of this study was to prepare O/W emulsions that can be used as potential treatment for cellulite, based on natural ingredients such as vegetable oils and butters, hydrolyzed collagen, caffeine, ivy extract and pink pepper essential oil, and to evaluate the rheological properties of these emulsions.

## 2. Materials and Methods

Type I collagen hydrolysate was obtained by acid hydrolysis of wet white leather wastes at 125°C during 8 hours according to the technology previously described [15].

Vegetable oils and butter (shea butter, cocoa butter, almond oil, rice oil,*Echium* oil), emulsifiers (glyceryl stearate; cetearylolivate and sorbitanolivate), floral waters (hamamelis water, immortelle water), xanthan gum, caffeine, ivy extract and preservative Cosgard (benzyl alcohol, salicylic acid, glycerin, sorbic acid) were purchased from a local pharmacy.

Pink pepper (*Schinusmolle.* L.) was collected in the mature period of seeds from Hatay Mustafa Kemal University botanical garden.

### 2.1. Essential oil extraction

The pink pepper essential oil was obtained by hydrodistillation using a Clevenger apparatus. Thus 600 g freshly grounded pink pepper was subjected to hydrodistillation for 3 hrs using 1000 mL of distilled water. The collected oil was allowed to stand for 30 min and then was dried over anhydrous sodium sulphate, stored in glass bottles and keep at 4 °C until use for analysis.

The oil extraction yield was calculated using the formula (1):

$$extraction\ yield\ (\%) = \frac{volume\ of\ oil\ (mL)}{plant\ material\ weight\ (g)} x100 \quad (1)$$

### 2.3. Formulation of O/W emulsions

The chemical composition of the O/W emulsions with potential anticelluliticaction is presented in Table 1:

*Table 1*

**Chemical composition of O/W emulsions with potential anticellulitic action**

| | | Emulsions | | | | | |
|---|---|---|---|---|---|---|---|
| **Phase** | **Ingredients** | **S1** | **S2** | **S3** | **S4** | **S5** | **S6** |
| **A** | shea butter, g | 15,8 | 10 | 5.8 | - | - | - |
| **A** | cacao butter, g | - | - | - | 15,8 | 10 | 5.8 |
| **A** | almond oil, mL | 10 | 10 | 5 | 10 | 10 | 5 |
| **A** | rice oil, mL | 10 | 5,8 | 5 | 10 | 5,8 | 5 |
| **A** | Echiumoil, mL | 5 | 5 | 5 | 5 | 5 | 5 |
| **A** | glyceryl stearate, g | 5 | 5 | 5 | - | - | - |
| **A** | cetearylolivate, sorbitanolivate, g | - | - | - | 5 | 5 | 5 |
| **B** | Water, mL | 25 | 30 | 35 | 25 | 30 | 35 |
| **B** | hamamelis water, mL | 25 | 30 | 35 | - | - | - |
| **B** | immortelle water, mL | - | - | - | 25 | 30 | 35 |
| **C** | collagen hydrolysate, g | 1 | 1 | 1 | 1 | 1 | 1 |
| **C** | xanthan gum, g | 0,2 | 0,2 | 0,2 | 0,2 | 0,2 | 0,2 |
| **C** | Caffeine, g | 2 | 2 | 2 | - | - | - |
| **C** | ivy extract, mL | - | - | - | 2 | 2 | 2 |
| **D** | preservative Cosgard, mL | 0,5 | 0,5 | 0,5 | 0,5 | 0,5 | 0,5 |
| **D** | pink pepper essential oil, mL | 0,5 | 0,5 | 0,5 | 0,5 | 0,5 | 0,5 |

The ingredients of phase A and phase B were heated in a water bath in two heat-resistant Berzelius beakers, periodically homogenizing the composition. When both phases reach a temperature of about 70-75 $^{\circ}$C, they were removed from the water bath; and phase B was slowly added over phase A under continuous stirring. The mixing continued for 10 minutes, avoiding as much as possible the aeration of the emulsion. The ingredients from phase C were then added to the composition and mixed for a few minutes. The beaker was placed in a cold-water bath under continuous stirring for 15 min. In the cooled composition, the ingredients of phase D are added and mixed slowly. The obtained emulsion is transferred to a sterile container.

### 2.4. Characterization of essential oil using GC-MS

Analysis of the pink pepper essential oil obtained was performed using a Thermo Scientific Focus gas chromatograph equipped with a mass spectrometer, autosampler and TR-5MS (5% phenyl-polisilfenilenesiloxan, 30 m x 0.25 mm inner diameter, film thickness 0.25). The carrier gas was helium (99.9%) with a flow rate of 1 mL/ min; ionization energy was 70 eV. Mass range m/z 50-650 amu. Data acquisition was scanning mode. Transfer line temperature of the mass

spectrometer was 220°C, the temperature of orifice injection was 220°C. The samples were injected with a split ratio of 250. The injection volume was 1 μL. The temperature of oven was programmed in the range of 50 to 220°C at 3 °C/min. The structure of each compound was identified by comparison of their mass spectra (Wiley 9 library). The data were processed using Xcalibur software.

### 2.5. Characterization of O/W emulsions

***Physical characterization, stability and pH;*** For the obtained emulsions the organoleptic (color, appearance, smell) and physical (phase separation) properties were optically observed. pH was evaluated using a inoLab pH-meter. Also, stability tests were performed at different temperatures: room temperature (20-23°C), 4-5 °C (refrigerator) and 40 °C (incubator) for all obtained emulsions.

***Optical microscopy analysis*** was performed using a LEICA optical microscope model S8AP0, with Increase Power: 20-160x. Samples were placed on glass slips and viewed through hot, transmitted light, which also has the option of filtering light using multiple lens types.

***Determination of water content and volatile substances;*** 1 g of samplewas weighed in a vial, placed into the oven at 100 ± 5 °C and dried to constant mass.The water content and volatile substances is calculated using equation (1):

$$water\ content\ and\ volatile\ substances\% = \frac{m2-m1}{m} x100 \quad (1)$$

where:
$m_2$- mass of the vial sample before drying, (g)
$m_1$- mass of the vial sample after draying, (g)
m- mass of the sample taken for analysis, (g)

***Rheological analysis*** was performed with a rotational rheometer Kinexus Pro (Malvern) with a Peltier element for a rigorous temperature control and a cone-plate geometry. The upper plate has a diameter of 40 mm and an inclination of 4°. The distance between the two plates was kept constant during the measurements at 0.15 mm. The samples were placed on the lower plate, and after the top plate was lowered, the excess was removed with a spatula. The tests were performed in the shear rate range $10^{-1} \div 10^{2}$ $s^{-1}$. The recorded data were represented in a logarithmic graphic. Measurements were performed at 24 °C (storage temperature) and 33°C (skin temperature).

## 3. Results and Discussion

### Essential oil extraction

The obtained pepper essential oil is clear, transparent, with characteristic pepper smell and an extraction yield of 1.135% (Volume of obtained oil = 6.81

mL). The obtained results correspond to the literature data, which shows a high purity of the obtained essential oil [16].

**Characterization of essential oil using GC-MS**

GC-MS chromatogram of pink pepper oil is shown in the Fig. 1:

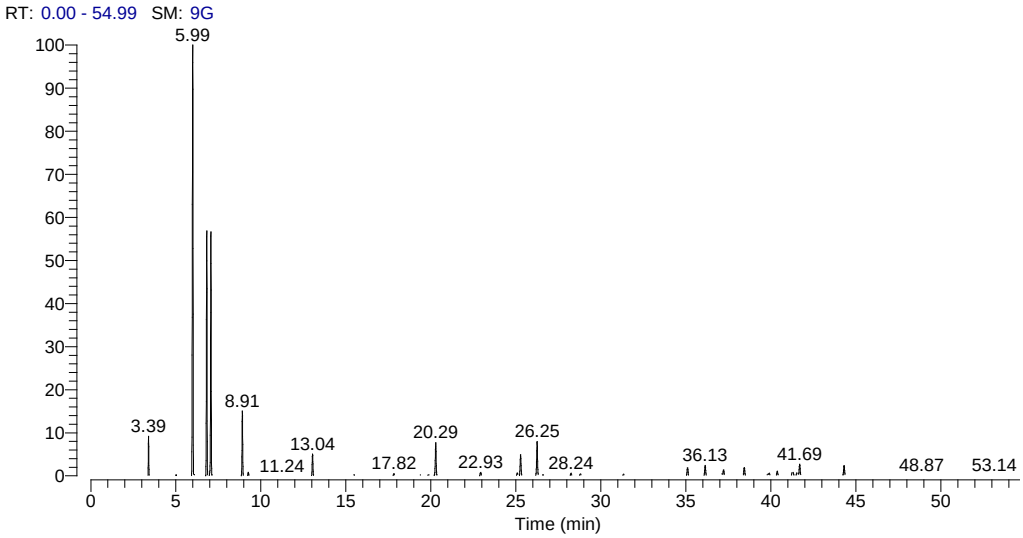


Fig. 1. GC-MS chromatogram of pink pepper essential oil

The chemical composition of pink pepper essential oil is shown in Table 2:

*Table 2*

**Chemical composition of pink pepper essential oil**

| RT, min | Compound name | Area % | RT, min | Compound name | Area % |
|---|---|---|---|---|---|
| **3.39** | **α-Pinene** | **1.98** | 26.60 | Neryl acetate | 0.16 |
| 5.01 | Sabinene | 0.07 | 28.24 | α-Phellandrene epoxide | 0.27 |
| **5.99** | **α-Phellandrene** | **35.84** | 28.79 | Geranylisovalerate | 0.21 |
| **6.82** | **Limonene** | **17.31** | 29.82 | Nerol | 0.09 |
| **8.91** | **o-Cimene** | **4.65** | 30.84 | Junipene | 0.07 |
| 9.26 | α-Terpinolene | 0.28 | 31.96 | Palustrol | 0.10 |
| **13.04** | **β-Phellandrene** | **13.04** | 32.59 | Ledene | 0.11 |
| 17.82 | α-Gurjunene | 0.23 | 35.10 | Caryophyllene oxide | 0.91 |
| 19.36 | 1-Terpineol | 0.12 | 35.28 | Globulol | 0.12 |
| 19.85 | Endobornyl acetate | 0.16 | 36.48 | α-Cedrene | 0.13 |
| **20.29** | **trans-Caryophyllene** | **3.04** | 37.10 | Veridiflorol | 0.30 |
| 20.63 | Alloaromadendrene | 0.12 | 37.22 | Elemol | 0.65 |
| 22.93 | α-Humulene | 0.36 | 37.62 | Geranylhexanoate | 0.11 |
| 23.04 | Cryptone | 0.07 | 38.43 | Spathulenol | 0.84 |
| 23.72 | Valencene | 0.10 | 39.81 | Guaiol | 0.15 |
| 24.38 | Germacrene | 0.09 | 40.38 | τ-Muurolol | 0.45 |

| | | | | | |
|---|---|---|---|---|---|
| 25.08 | α-Muurolene | 0.30 | 41.27 | Guaiol | 0.60 |
| **25.28** | **Bicyclogermacrene** | **2.13** | 41.54 | α-Eudesmol | 0.54 |
| 25.49 | Neryl acetate | 0.10 | 41.69 | α-Cadinol | 1.21 |
| **26.25** | **δ -Cadinene** | **3.27** | 44.31 | Globulol | 1.09 |
| | | | | **Total** | **91.37** |

The main components of pink pepper essential oil are: α-Phelandrene 35.84%, Limonene 17.31%, α-Pinene 1.98% (monoterpenes) and β-Phelandrene 13.04%, o-Cymene 4.65%, δ-Cadinene3.27%, trans-Caryophyllene 3.04%, Bicyclogermacrene 2.13%(sesquiterpenes).

In comparison to other studies involving the volatile oil of pink pepper, monoterpenes were found the main constituents of the oil (58.9%), including α-pinene (31.1%) and β-pinene (22.7%) as the main compounds. The second group in quantity was constituted by sesquiterpenes (29.9%), with Cadinene (6.0%), epi-α-cadinol (5.6%) and β-caryophyllene (4.7%) as the major constituents [17].

The differences from the data specified in the literature for pink pepper essential oil can be generated by a number of factors such as soil and climatic conditions, time of harvest, subspecies, and differences related to the extraction method applied (different parameters).

## Characterization of O/W emulsions

### *Physical characterization, stability and pH*

All the emulsions obtained are homogeneous and have a white color, as shown in Fig. 2:

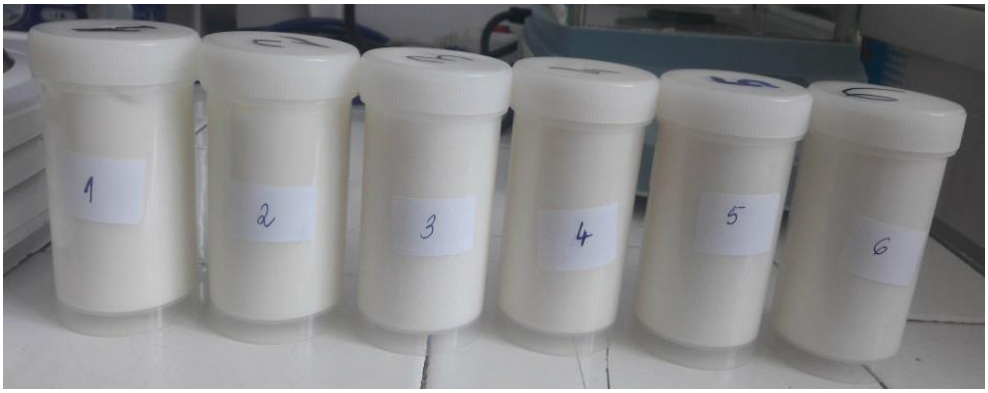


Fig. 2. O/W emulsions with a potential anticellulitic action

All emulsions have a pleasant, creamy, moisturized appearance and a smell specific to the essential oil in the composition (pink pepper). No phase separation was observed shortly after preparation. Following the stability tests, all emulsions were found to be stable at all three working temperatures.

The pH values of the obtained emulsions are shown in Table 3:

*Table 3*

**The pH values for the obtained emulsions**

| | S1 | S2 | S3 | S4 | S5 | S6 |
|---|---|---|---|---|---|---|
| **pH** | 6.0 | 6.0 | 6.0 | 5.5 | 5.5 | 5.5 |

The natural pH of the skin varies between 4.5-6.5 and this interval is considered to be optimal for cosmetic products that are indirect contact with the skin [18]. The obtained values for the emulsions are in this interval, indicating that emulsions can be safely applied on the skin.

***Optical microscopy analysis*** results are presented in Fig. 3:

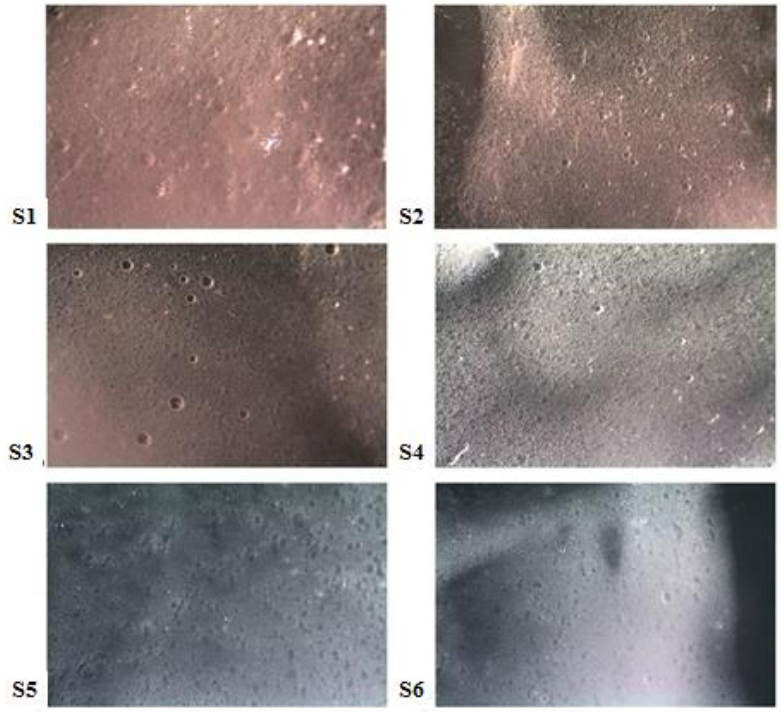


Fig. 3. Images obtained by optical microscopy for emulsion variants S1-S6, magnification power 32 x

In Fig. 3 it can be seen that the oil droplets are dispersed uniformly (S1, S4, S6) or less unifrom (S2, S3, S5), also the particle size varies from one sample to another, the content of vegetable oils in different proportions being responsible for these differences due to the fatty acids (oleic, linoleic, palmitic, etc.). Also, differences in the type of emulsifier used can be observed, as follows: emulsions S1-S3 (obtained with the glyceryl stearate emulsifier) have a creamier appearance

and emulsions S4-S6 (obtained with the cetearylolivate and sorbitanolivate emulsifier) have a "foam" slightly aerated appearance.

***Determination of water content and volatile substances***

Water content and volatile substance are presented in Table 4:

*Table 4*

**The value for water content and volatile substance for the obtained emulsions**

| | **S1** | **S2** | **S3** | **S4** | **S5** | **S6** |
|---|---|---|---|---|---|---|
| **m (g)** | 1.064 | 1.058 | 1.063 | 1.068 | 1.045 | 1.053 |
| **$m_1$ (g)** | 8.203 | 8.320 | 8.226 | 8.150 | 8.217 | 8.218 |
| **$m_2$ (g)** | 8.709 | 8.934 | 8.940 | 8.646 | 8.845 | 8.956 |
| **water content and volatile substance, %** | 47.556 | 58.034 | 67.168 | 46.441 | 60.095 | 70.085 |

Table 4 shows that the most hydrated emulsions are S3 and S6 with the highest content of water and volatile substances, respectively 67.17% and 70.08%.

***Rheological analysis***

The results of the rheological experiments obtained at two operating temperatures (24and 33˚C) for the designed cosmetic emulsions, are presented in Figs. 4, 5:

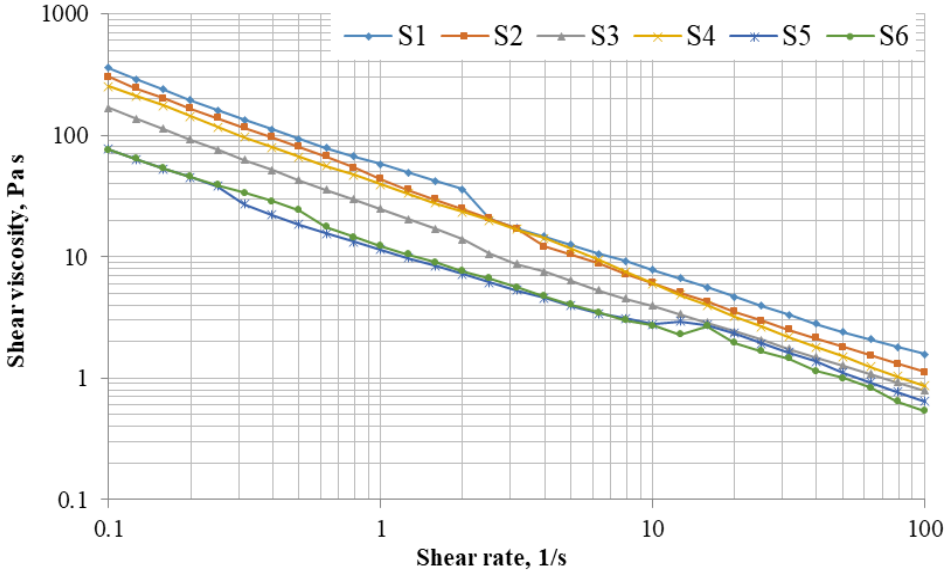


Fig.4: Flow behavior of the synthesized compositions, registered at 24˚C

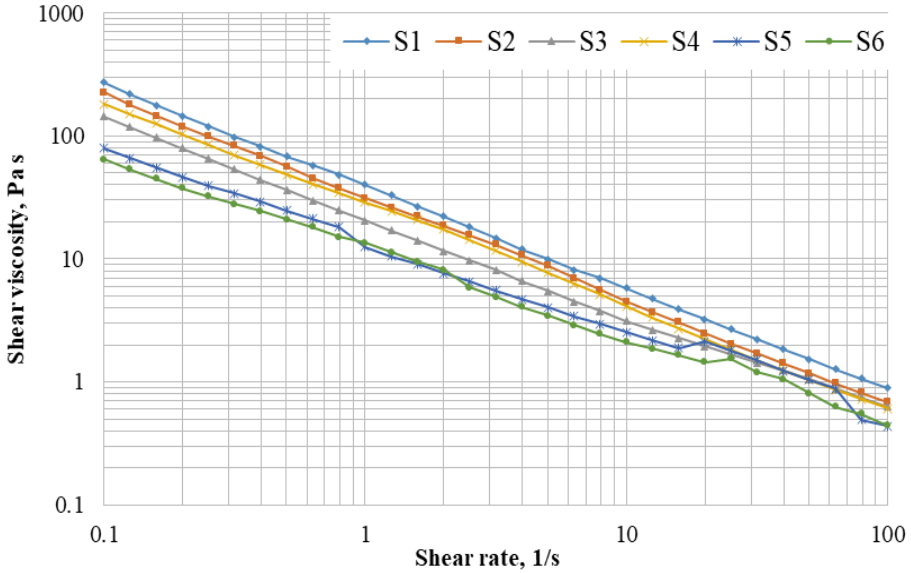


Fig.5: Flow behavior of the synthesized compositions, registered at 33˚C

The rheology tests showed that all synthesized compositions have a shear thinning behavior, at both storage and skin temperature (24 and 33˚C, respectively). As expected, the values registered for the viscosity at 24˚C are slightly higher for all compositions with respect to the ones registered at 33˚C. For example, the shear viscosity registered at 24˚C for P1 is 356,5 Pa s while the shear viscosity registered at 33˚C for the same sample is 225 Pa s. Moreover, regardless of the testing temperature, the difference between the viscosities of different cream compositions is higher at lower shear rates than at high shear rates, which results in a similar spreadability for all compositions.

## 4. Conclusions

In this study, we prepared some O/W emulsion formulations using collagen hydrolysate (the most important protein in the skin), mixtures of vegetable oils, pink pepper essential oil, caffeine, ivy extract (with anticelullitic action) and other natural ingredients.

The 6 obtained emulsions are stable at different temperatures and the value obtained for the pH of the emulsions correspond to the natural pH of the skin, indicating that emulsions can be safely applied to the skin. The most hydrated emulsions are S3 and S6 with the highest content of water and volatile substances.

Results of the optical microscopy analysis shows that emulsions S1-S3 (obtained with the glyceryl stearate emulsifier) have a creamier appearance and emulsions no. S4-S6 (obtained with the cetearylolivate and sorbitanolivate emulsifier) have a " foam" slightly aerated appearance.

The rheological tests confirmed the shear thinning behavior of the synthesized cream compositions, meaning that the viscosity is not independent of shear rate, but the creams became less viscous when higher shear rates are applied. Moreover, comparing the viscosity values registered at low (0.1 $s^{-1}$) and high ($10^2$ $s^{-1}$) shear rated it can be concluded that all compositions have a better spreadability when the shear rate is increased.

It can be concluded that all the emulsions prepared are stable, safe for the skin and present adequate rheological properties, so it can be used as a natural alternative for the treatment of cellulite. In the future microbiological tests and others analysis are necessary for the obtained emulsions.

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