Essential mint oil-based emulsions: preparation and characterization

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REZUMAT – ABSTRACT

Emulsii obţinute pe bază de ulei esenţial de mentă: obţinere şi caracterizare

Scopul acestei lucrări este de a prezenta pe scurt metodologia de obţinere a patru tipuri de emulsii (M_2, M_3, M_6 şi M_7) pe bază de ulei esenţial de mentă (Mentha Piperita) şi proprietăţile fizico-chimice şi calitative ale acestora (pH, densitate, indice de aciditate, indice de peroxid, conţinutul de diene şi triene conjugate, stabilitatea în timp, conţinutul de apă şi materie grasă), cu scopul de a selecta varianta optimă de formulare a emulsiei pentru aplicare în domeniul textil. Această lucrare subliniază, de asemenea, faptul că cea mai stabilă este emulsia M_6 urmată de emulsia M_3.

Cuvinte-cheie: ulei esenţial de mentă, ceară de albine, emulsie, proprietăţi fizico-chimice şi calitative

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The aim of this work is to present briefly the preparation methodology of four emulsions (named M_2, M_3, M_6, and M_7) based on extracted mint oil (Mentha Piperita) and their physical-chemical properties and quality characteristics (pH, density, acidity index, peroxide index, diene and triene content, in-time stability, humidity and fatty matter content), in order to select the most recommendable emulsion to be used in textile field. This work also underlines that the most stable emulsion is M_6 emulsion followed by M_3 emulsion.

Keywords: mint essential oil, beeswax, emulsion; physical-chemical properties; quality characteristics

INTRODUCTION

Researches from last years have shown that aromatherapy textiles are increasingly used because of the benefits offered as: environmentally friendly and beneficial effects on wellbeing and human health.

Essential oils are a common source of bioactive ingredients and are used for their various functional properties (anti-inflammatory, anti-microbial, organoleptic, antioxidant, anti-arrhythmic, anti-thrombotic, anti-vasoconstrictive, anti-hypertension, anti-ulcerous, anti-aging, anti-carcinogen, anti-diabetic, anti-depressant, anti-pyretic, and insect repellent) [1]. In textile field, most essential oils are used for their antimicrobial effect [2].

Although skin is not a common way of managing biologically active principles due to difficult absorption especially for water-soluble substances, in recent years, more attention has been paid to the benefits of transdermal administration: controlled release of biologically active principles leading to reduced invasiveness; reduction of the metabolic stages in the liver; avoiding stomach trauma [3, 4].

Losses by evaporation and difficulties in controlling releases make the application of essential oils to be limited. In this case, carrier systems (lipid particles, nano-emulsions, biocompatible polymer particles) can provide an ideal solution to achieve a controlled and targeted distribution of essential oil.

Natural waxes are suitable for essential oils embedding [5]. Also, natural waxes are of food grade purity (insect waxes – bees wax and plant waxes – candelilla wax, carnauba wax) and exhibit interesting rheology and microstructure [5].

In last years have been reported a series of researches on successful applications of beeswax in preparing diverse biodegradable films and coatings [6].

Bees wax is soft, biodegradable and a considerable viable absorbent. It is used to create a cover around the materials as a hydrophobic layer [7].

The study aims to produce the beeswax/mint essential oil emulsions and to analyze a few of its physical-chemical and quality characteristics.

EXPERIMENTAL PART

Materials and methods

▪ *Basic materials for emulsions preparation: raw and auxiliary materials*

For all experimental researches, it was used as basic raw matter the essential mint oil received from the Turkish company Doğal Destek.

As auxiliary materials, there were used: (*i*) beeswax purchased from a private apiary in the Northeast region of Romania; (*ii*) glycerin purchased from SC Elemental SRL, Romania, and (*iii*) Tween 80 (Merck, Germany).

All other chemical reagents used for analytical analysis were of analytical purity (p.a.), being purchased from Romanian companies (e.g., Chemical Company S.A., Iasi, RO), or from abroad (Sigma Co., or Merck Co.).

▪ *Emulsion preparation methodology*
The beeswax was melted with a 700 rpm rate at a temperature of 65°C, over which distilled water was added at 63°C to form the wax/water system. Tween 80 emulsifier was added to the wax/water system, and the system was maintained under stirring for 10 minutes at 63°C, after which glycerol was added. After complete homogenization, the system was cooled to 40°C, over which the mint essential oil was added dropwise.There were prepared series of emulsions (M_i) which are different due to varying ratios of wax/essential oil used (there were selected as representative series – M_2, M_3, M_6 and M_7 emulsions having the ratio wax/oil varying in range of 1:2; 1:1.3; 1:5; and 1:6).

The prepared esential mint oil-based emulsions were analyzed and characterized further especially for its stability and transformation degree.

Analysis methods

▪ *Emulsions appearance*
The appearance of the four prepared emulsions was visually and microscopically analyzed using a KRÜSS optical microscope equipped with a NIKON Coolpix P 5100 photo digital camera.

▪ *pH determination*
Emulsion *pH* was directly measured using a HANNA portable pH-meter immersed in the prepared non-diluted emulsion (M_i).

▪ *Density determination*
All measurements were performed directly using an Anton Paar DMA 4500 Density Meter (Anton Paar GmbH, Granz, Austria) at three different temperatures of 19°, 20° and 25°C. For each temperature, there were performed at least six till eleven measurements and calculated the mean value of density at specific temperature. This mean value was reported in our work.

▪ *Determination of the acidity index (AI)*
Around 2.5 g of emulsion sample (weighted with precision of 0.001 g) was contacted with 12.5 mL of chloroform and 12.5 mL of ethylic alcohol. After stirring, there were added a few drops of phenolphthalein indicator and the obtained solution was titrated under stirring with potassium hydroxide (0.1 M KOH) till a pink color obtained, stable at least 1 min. For calculation of the acidity index (*AI*) it was used the relation (1):

$$AI = \frac{V_{KOH} \cdot M_{KOH} \cdot 56.11}{m} \left[\frac{\text{mg of KOH}}{\text{g of emulsion}} \right] \quad (1)$$

where: *AI* – the acidity index (mg KOH/g of emulsion); V_{KOH} – the volume of KOH consumed at titration (mL); M_{KOH} – the concentration of KOH solution (mol/L); 56.11 – molecular weight of KOH (g/mol) and *m* – sample mass (g).

▪ *Determination of the peroxide index (PI)*
In a closed glass vessel was weighed around 1–2 g of emulsion which was contacted with 5 mL of chloroform, 7.5 mL of glacial acetic acid and 1 mL of 10% KI. The closed vessel was stirred for 1 min and after set in a dark place for 15 min. It was added 37.5 mL of distilled water and, after stirring, was introduced starch solution till a dark stable blue color appears. The formed iodine is titrated with sodium thiosulfate (0.05 N $Na_2S_2O_3$). A control titration is performed in parallel with the basic determination. For calculation of the peroxide index (*PI*) it was considered the following relation:

$$PI = \frac{(V_{ref} - V) \cdot 1000}{m} \left[\frac{\text{mmol of peroxide}}{\text{g of emulsion}} \right] \quad (2)$$

where: *PI* is the peroxide index (mol of peroxide/kg of emulsion), V_{ref} – volume of $Na_2S_2O_3$ solution consumed at titration of control sample (mL), *V* – volume of $Na_2S_2O_3$ solution consumed at titration of analyzed emulsion sample (mL), $N_{Na_2S_2O_3}$ – normal concentration of sodium thiosulfate solution (val/L), 1000 – recalculation coefficient of [mol of peroxide/g] in [mol of peroxide/kg] and *m* – sample mass (g).

▪ *Determination of conjugated diene or triene concentration*
The method is based on the absorbance measurement at a fixed wavelength in UV field for a constant mass of emulsion sample, *i.e.* 236, or 267 nm for diene and 273 nm for conjugated triene [8]. A emulsion sample of 0.1 g was diluted with distilled water till 25 mL in a volumetric flask. The absorbance measurement of emulsion sample was performed at Camspecspectrophotometer M 500. The value for conjugated diene (CD) and triene (CT) concentration is calculated as in relation (3) or (4):

$$C_{CD} = \frac{A_{236/267} \cdot (2.5 \cdot 10^4)}{(\varepsilon \cdot l) / m} \quad (3)$$

$$C_{CT} = \frac{A_{273} \cdot (2.5 \cdot 10^4)}{(\varepsilon \cdot l) / m} \quad (4)$$

where: C_{CD} – molar concentration of conjugated diene (mol/kg, or mmol/g of emulsion), C_{CT} – molar concentration of conjugated triene (mmol/cm^{-3}), $A_{236/267}$ or A_{273} – absorbance of diluted emulsion at 236, or 267 nm, and 273 nm, ε – molar absorbance (extinction coefficient) for linoleic acid hydroperoxide ($\varepsilon = 2.525 \cdot 10^4$ M^{-1}·cm^{-3}), *l* – cuvette length (l = 1 cm) and *m* – sample mass (g).

RESULTS AND DISCUSSIONS

Emulsion appearance

Emulsions appearance is presented in figure 1.
According to microscopic images, the dispersed molecule phase is presented as a compact, dense small globule mass. Related to visual images, emulsions M_2 and M_3 are homogeneous, white, without agglomerations of particles and easily handle. Emulsions M_6 and M_7 are translucent and free of particles in suspension.
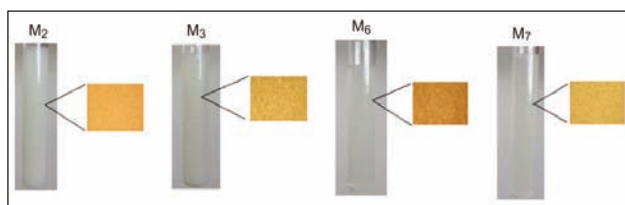
Fig. 1. Emulsions appearance

Physical-chemical analysis of emulsions

The physical-chemical properties of emulsions play a special role in preparation and further utilization of M_i emulsions based on mint essential oil and are dependent of the chemical and structural composition. Therefore, there are necessary serious investigations for selection of a few emulsion compositions with increased value and physical, oxidative and microbiological stability. In this context, there were determined a few basic quality indices of prepared M_i emulsions according with the international approved standard norms for cosmetic and food products.

The quality indices were determined at room temperature (19–22°C) during the period of emulsions storage for at least 4 weeks.

The selection of emulsion composition was performed for obtaining an optimal ratio of poly unsaturated fatty acids in triglycerides with curative-prophylactic properties, good application on textile fabrics and assurance of emulsion resistance to different oxidations and also obtaining of an acceptable acidity (aggression) on contact with skin.

In table 1 are presented some values of physical-chemical quality indicators of the investigated M_i emulsions.

The general physical-chemical quality indicators of the four investigated M_i emulsions were analyzed being in range of 67.26–78.9% for separated aqueous phase, 21.21–32.26 % for separated fatty phase (organic phase), 3.5–4.9 for pH, 1.0204–1.0310 g/cm^3 for average absolute density at 19°C, 1.0193–1.0307 g/cm^3 for mean density at 20°C and 1.0171–1.0299 g/cm^3 for mean density at 25°C. All these characteristics are corresponding to the standardized norms in cosmetic products.

During the storage period, the prepared M_i emulsions are supposed to possible degradation processes which can affect their quality. Usually, the emulsions can be slowly oxidized and converted in a complex organic system with a high number of components due to all oxidation steps (*i.e.* initiation, development and breaking of different macromolecular chains).

To analyze the in-time variation of prepared emulsions' quality is one of the important aim of this work. That is why it is important to establish the dynamic of primary oxidation products formed (*i.e.* hydroperoxides) during the storage of M_i emulsions, considering especially the acidity index (*AI*), peroxide index (*PI*), conjugated diene (*CD*) and triene (*CT*) concentrations. The analysis is performed for a period of 4 weeks at room temperature (19–23°C), in absence of light only in the night period.

The experimental results are summarized in table 2. In period of emulsions storage can take place the oxidative and hydrolytic degradation which is characterized by formation of oxidation and hydrolysis products, expressed by the value of free fatty acids or fatty phase separation, and acidity index.

The value of acidity index in the four investigated M_i emulsions was varied in 4 weeks between 1.121 and

<div align="right">Table 1</div>

SOME PHYSICAL-CHEMICAL QUALITY INDICES OF INVESTIGATED EMULSIONS

Physical-chemical quality indicators	M_2	M_3	M_6	M_7
Aqueous phase, [%]	78.79	69.70	67.26	77.50
Fattyphase (organic phase), [%]	21.21	30.30	32.26	22.50
pH (room temperature, t = 23.7°C)	3.5±0.3	4.8±0.2	5.0±0.2	4.9±0.2
Density (absolute, mean value), [g/cm^3]				
19°C	1.0310	1.0294	1.0204	1.0252
20°C	1.0307	1.0294	1.0193	1.0240
25°C	1.0299	1.0283	1.0171	1.0225
Acidity index, [mg KOH/g of emulsion]	1.1420	2.2716	0.8672	0.8944
Peroxide index, [mmol/g of emulsion]	6.7895	6.1627	5.7306	2.8020
Diene concentration, [μmol/g of emulsion]	2.998	9.370	3.840	1.856
Conjugated triene concentration, [μmol/g]	2.539	8.125	2.674	1.248
Stability in a month, [%]				
1-3 days	Stable	Stable	Stable	Stable
4 days	78.89	Stable	Stable	78.89
1 week	50.00	69.70	Stable	50.00
2 weeks	31.30	50.00	Stable	30.30
3 weeks	21.21	30.21	Stable	22.21

Table 2

ACCUMULATION OF PRIMARY LIPIDIC OXIDATION PRODUCTS IN PERIOD OF EMULSIONS STORAGE					
Physical-chemical quality indicators	Storage period	M_2	M_3	M_6	M_7
Acidity index (*AI*), [mg KOH/g of emulsion]	1 week	1.121	2.263	0.862	0.885
	2 weeks	1.123	2.268	0.863	0.889
	3 weeks	1.136	2.270	0.865	0.892
	4 weeks	1.142	2.272	0.867	0.894
Peroxide index (*PI*), [mmol/g of emulsion]	1 week	6.7884	6.1611	5.7297	2.7002
	2 weeks	6.7890	6.1613	5.7298	2.8005
	3 weeks	6.7894	6.1625	5.7306	2.8018
	4 weeks	6.7895	6.1627	5.7306	2.8020
Diene concentration (*CD*), [µmol/g of emulsion]	1 week	2.686	9.302	3.834	1.611
	2 weeks	2.712	9.325	3.836	1.618
	3 weeks	2.834	9.354	3.841	1.787
	4 weeks	2.998	9.370	3.840	1.856
Conjugated triene concentration (*CT*), [µmol/g of emulsion]	1 week	2.358	8.109	2.645	1.104
	2 weeks	2.404	8.118	2.659	1.116
	3 weeks	2.512	8.123	2.668	1.203
	4 weeks	2.539	8.125	2.674	1.248

1.142 mg KOH/g of emulsion for M_1, 2.263 and 2.272 mg KOH/g of emulsion for M_2, 0.862 and 0.867 mg KOH/g of emulsion for M_6 and 0.885 and 0.895 mg KOH/g of emulsion for M_7. Analyzing these AI_i values of prepared mint oil-based M_i emulsions in the period of their storage at room temperature, it can conclude that all AI_i values increase, fact which demonstrates the accumulation of free fatty acids. The values of acidity indices (AI_i) differ significantly, the highest being obtained for M_3 emulsion and the lowest for M_7. In addition, the fatty phase separated was the highest in the case of M_7 emulsion followed by M_3 emulsion. This fact can be explained by the high content of fatty acids with double and triple bonds, which are degraded much faster in the storage activity (at room temperature).

It is known that the presence of peroxide (hydroperoxide) in emulsion determines the degree of emulsion stability during the storage process (at room temperature). The values of peroxide index were not significantly changed after 4 weeks, varying for M_2 emulsion in range of 6.7884–6.7895 mmol/g, 6.1611–6.1627 mmol/g for M_3 emulsion, 5.7297–5.7306 mmol/g for M_6 emulsion and 2.7002–2.8020 mmol/g for M_7 emulsion. Based on the resulted experimental data, the highest peroxide concentration was performed in the case of M_2 which was found also with lowest stability in time at room temperature, followed by M_7 emulsion.

For the study of lipids oxidation, it must be analyzed the variation of conjugated diene and triene concentration which are produced from unsaturated fatty acids as result of hydroperoxide formation due to rearrangements of double bonds. The formed conjugated diene presents an intense absorption at the wave length of 234 nm or 267 nm, and in case of triene the intense absorption is registered at 273 nm. An increase of UV absorption indicates the formation of primary oxidation products in the investigated M_i emulsions. This fact concludes clearly that the storage

of M_i emulsion at room temperature acts in direction of increasing the conjugated diene and triene concentrations. The highest value during 4 weeks of storage at room temperature was achieved for M_3 emulsion, *i.e.* 9.303–9.370 µmol/g of emulsion for diene concentration and 8.109–8.125 µmol/g of emulsion for triene concentration, and the lowest for M_7 emulsion, *i.e.* 1.611–1.856 µmol/g of emulsion for the diene concentration and 1.104–1.248 µmol/g of emulsion for the triene content.

In addition, the peroxides are instable compounds, but in the process of emulsion storage these can be decomposed forming secondary oxidation products as aldehydes, ketones and its derivates with carbonyl chain of different lengths. The peroxides have no direct influence on sensory indices of emulsions, but if aldehydes and ketones are formed, these have rancid odor. In the case of M_i emulsions it was found no such a rancid odor, being indicated that secondary oxidation products were not formed during the period of 4 weeks of emulsion storage at room temperature.

CONCLUSIONS

The physical-chemical quality indices of investigated M_i emulsions based on mint essential oil were indicated that the highest stability had the M_6 emulsion followed by M_3 emulsion.

The comparative analysis of experimental results performed for the acidity index, peroxide index, conjugated diene and triene concentration were permitted some preliminary information concerning the dynamic of M_i emulsion degradation during the storage activity at room temperature for a time period of 4 weeks. The results indicated the formation of primary oxidation products (hydroperoxides), but no formation of secondary oxidation products (aldehydes and ketones) with their specific rancid odor.

The general physical-chemical indicators of investigated M_i emulsions were found corresponding with

the standardized norms in cosmetics and textiles for topical applications.

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