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EMULSIONS BASED ON ROSEMARY ESSENTIAL OIL – BEESWAX SYSTEM USED IN MANUFACTURING OF VALUE – ADDED TEXTILES FOR SKIN CARE BENEFITS

BY

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Abstract. This work presents briefly the preparation methodology of nine emulsions based on beeswax – rosemary essential oil system (*iR*, *i*=1…9) and its specific quality characteristics, representatives being emulsions 3*R*, 4*R*, 8*R* and 9*R* in manufacturing of value – added textiles for aromatherapy and skin care benefits. These prepared emulsions were preliminarily characterized by some specific physical-chemical properties and quality indicators (*i.e.* pH, absolute density, acidity index, peroxide index, content of conjugated dienes and trienes, total content of polyphenols and flavonoids) as well as sensory analysis which was permitted the recommendation of the most indicated emulsion to be used by a Romanian textiles manufacturer in order to add value to its textile products, considering emulsions in-time stability (after 1 and 8 months of storage at room temperature) and its potential antibacterial action after textile impregnation.

This work underlines also that the most recommendable emulsion is 4*R* emulsion followed by 8*R* emulsion, both having a relative good in-time stability till the separation of organic and aqueous phases as well as a satisfactory content of polyphenols and flavonoids.

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Keywords: added-value textile material; emulsion; in-time stability; physical-chemical quality indicators; skin care benefit; textile impregnation.

1. Introduction

Commonly, for manufacturing of value-added textiles for aromatherapy and skin care benefits, the oil/water (O/W) type emulsions are preferred due to their better dispersion on the textiles as well as avoidance of oily sensation which appears after application of the product (Dănilă *et al*., 2019; Radu *et al*., 2017; Zaharia *et al*., 2018; Zaharia *et al*., 2019a; Zaharia *et al*., 2019b).

The main benefits offered by impregnated textiles with O/W emulsions are related to the beneficial effects of wellness state and human health, environmental-friendly behavior, and also its antibacterial action.

As preferred oils for preparation of such O/W emulsions are considered especially the vegetal essential oils, *e.g*. rosemary essential oil.

The essential oils (EOs) have been used in folk medicine due to their antimicrobial activity mainly dependent on their chemical composition (Dănilă *et al*., 2019; Zaharia *et al*., 2018; Zaharia, 2019a; Zaharia, 2019b). EOs act to inhibit the growth of bacterial cells and also inhibit the production of toxic bacterial metabolites. Therefore, it is necessary to formulate essential oils in various forms: liquid (emulsions), semi-liquid (gels), or solid (microcapsules or microspheres) for the controlled release of active compounds and their protection from the external environment, caused the biological EO activity may be lost by volatilizing, or degradation under the action of high temperature, oxidation and UV light (Dănilă *et al*., 2019; Radu *et al*., 2017; Zaharia *et al*., 2018; Zaharia *et al*., 2019a; Zaharia *et al*., 2019b).

The aim of this study is to present how it was developed a stable emulsion in which the oily phase is made up of beeswax mixed with rosemary essential oil, and to empirical design the influence of two operating variables onto emulsion characteristics, *i.e.* beeswax concentration and the essential oil content. Moreover, the study of in-time stability of prepared rosemary oil-based emulsions reffering to a period higher than 8 months at room temperature and also its sensory analysis are presented and discussed.

2. Experimental

2.1. Materials and Reagents

Beeswax was used as shall material for essential oil core, being procured from a private apiary in the Northern-Eastern region of Romania. Beeswax contains a large number of chemical compounds (> 300), which can be grouped according to Table 1 (Dănilă *et al*., 2019).

Rosemary essential oil (*R*) was purchased from the Turkish contract partner DOĞAL DESTEK, sub-contractor EÜ.

The emulsifying agent, Tween 80 ($C_{32}H_{60}O_{10}$), was supplied by Merck, Germany.

The agent for humidity preservation, 99.5% pure vegetable glycerin, was purchased from SC Elemental SRL Co., Romania.

Table 1

The Beeswax Composition with Different Chemical Compounds

Groups of contained compounds	[%]
Fatty acids (24-32 carbon atoms in the hydrocarbon chain)	12 - 14
Monoesters and hydroxyl iminoesters of palmitic acid, oleic acid (40-48 carbon atoms in the hydrocarbon chain)	35 - 45
Hydrocarbons (27-33 carbon atoms in the hydrocarbon chain)	12 - 16
Primary and free alcohols (28-35 carbon atoms in the hydrocarbon chain)	1

All other chemical reagents used in different analysis methods are of analytical purity (p.a.), *e.g.*, chloroform ($CHCl_3$), potasium hydroxide (KOH), phenolphthalein indicator, nitric acid (HNO_3), chloride acid (HCl) and sodium carbonate (Na_2CO_3), sodium tiosulphate ($Na_2S_2O_3$), methanol, ethanol, and also different prepared solutions (10% KI, 1% starch solution, standard quercitin, Folin-Ciocalteu solution) which were purchased from Romanian companies (Chemical Company S.A., Iași, RO), or from abroad (Sigma Co., or Merck Co.).

2.2. Analysis Methods

The main analysis methods considered in this research work are for the determination of emulsion pH, density, acidity index, peroxide index, content of conjugated dienes and trienes, total polyphenols, and total flavonoids, but also emulsions sensory analysis.

PH determination. The pH of prepared emulsions was directly measured using a HANNA high precision KL-009(I) pH-meter immersed in the prepared non-diluted emulsion (*iR*) (Mureşan *et al*., 2018).

Density determination. The density measurement was performed directly on an Anton Paar DMA 4500 Density Meter (Anton Paar GmbH, Granz, Austria) at standard temperature of 20°C. For each emulsion, there were performed at least six till ten measurements and calculated the mean value of density. This mean value is reported in this research work (Dănilă *et al*., 2019).

Determination of the acidity index (*AI*). Around 1.0 g of emulsion sample (weighted with precision of 0.001 g) was contacted with 5 mL of chloroform and 5 mL of ethylic alcohol. After stirring, it was added a few drops of phenolphthalein indicator and the obtained solution was titrated with potassium

hydroxide (0.01 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):

$$\boldsymbol{AI} = [(V_{KOH} \cdot M_{KOH} \cdot 56.11) / m], \text{ [mg of KOH / g of emulsion]} \quad (1)$$

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 – the molecular weight of KOH, [g/mol] and *m* – the sample mass, [g] (Capcanari, 2012; Dănilă *et al*., 2019; Zaharia *et al*., 2018; Zaharia *et al*., 2019a; Zaharia *et al*., 2019b).

Determination of the peroxide index (*PI*). In a closed glass bottle 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 bottle 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 1% starch solution till a dark stable blue color appeared. The formed iodine was 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 (Dănilă *et al*., 2019; Zaharia *et al*., 2018; Zaharia *et al*., 2019a; Zaharia *et al*., 2019b):

$$PI = \{[(V_{ref} - V) \cdot N_{Na2S2O3} \cdot 1000] / m\}, \text{ [mmol of peroxide / g of emulsion]} \quad (2)$$

where: *PI* – the peroxide index, [mmol of peroxide / g of emulsion]; V_{ref} – the volume of $Na_2S_2O_3$ solution consumed at titration of control sample, [mL]; *V* – the volume of $Na_2S_2O_3$ solution consumed at titration of analyzed emulsion sample, [mL]; $N_{Na2S2O3}$ – the normal concentration of sodium thiosulfate solution, [val/L]; 1000 – the recalculation coefficient of [mol of peroxide / g] in [mmol of peroxide / g] and *m* – sample mass, [g].

Determination of conjugated dienes or trienes concentration. The method is based on the absorbance measurement at a fixed wavelength in UV light range for a constant mass of emulsion sample, *i.e.* 236 nm for dienes and 273 nm for conjugated trienes (Capcanari, 2012; Dănilă *et al*., 2019; Zaharia *et al*., 2018; Zaharia *et al*., 2019a; Zaharia *et al*., 2019b). 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 the Camspec M500 spectrophotometer. The value for total conjugated dienes (CD) and trienes (CT) concentration is calculated as in relation (3) or (4):

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

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

where: C_{CD} – the molar concentration of total conjugated diene, [mol/kg, or mmol/g of emulsion]; C_{CT} – the molar concentration of total conjugated triene, [mmol/cm^{-3}]; A_{236} or A_{273} – the absorbance of diluted emulsion at 236, and 273 nm; ε – the molar absorbance (extinction coefficient) for linoleic acid hydroperoxide [$\varepsilon = 2.525 \cdot 10^4$ M$^{-1} \cdot$ cm^{-3}]; l – the cuvette length [l = 1 cm] and m – the sample mass, [g] (Capcanari, 2012).

Determination of total polyphenols content. It was used the spectrophotometer-based Singleton method with Folin-Ciocalteu reagent. 0.5 mL of emulsion was introduced in 10 mL of distilled water and, after sample stirring, was added 0.5 mL of Folin-Ciocalteu reagent. After 5 min, it was introduced 8 mL of 7.5% Na_2CO_3, and after 2 h it was measured the absorbance of treated sample at 765 nm under a blank with distilled water at SP 830 Plus spectrophotometer (MeterTech Inc.). If the absorbance value was higher than 1.8, the sample was diluted in ratios of 1:1, 1:2, 1:3, or 1:4. The total content of polyphenols was expressed using the calibration curve of gallic acid (selected model due to its stability and purity), in range of 0.05-0.5 mg/mL. The linear calibration equation (*i.e.* after experimental data processing by linear regression methodology) corresponds to the relation: y = 2.1169x – 0.0831, where *y* is the absorbance value measured at 765 nm (A_{765}) and *x* is the concentration of gallic acid, [μg/mL] (Lamien-Meda *et al*., 2005; Pontis *et al*., 2014; Zaharia *et al*., 2018; Zaharia *et al*., 2019a; Zaharia *et al*., 2019b).

Determination of total flavonoids content. 2 mL of extract/emulsion was treated with 2 mL of $AlCl_3$ dissolved in 2% methanol. The sample was placed in dark room for 10 min, and after it was measured its absorbance at 510 nm under a blank composed of 1 mL of methanol and 1 mL of $AlCl_3$ 2% at SP 830 Plus spectrophotometer (MeterTech Inc.). The total content of flavonoids was expressed using the standard calibration curve of quercetin (selected model due to its stability and purity), in range of 0.005-1.2 mg/mL. The linear calibration equation (*i.e.* after experimental data processing by linear regression methodology) corresponds to the relation: y = 0.0005x – 0.037, where *y* is the absorbance value measured at 510 nm (A_{510}) and *x* is the concentration of quercetin [μg of quercetin equivalent (QE)/mL]. The total content can be expressed also in [μg of quercetin equivalent (QE) / g of emulsion] if are known the emulsion volume, [mL] and its weight, [g]) (Lamien-Meda *et al*., 2005; Pontis *et al*., 2014; Zaharia *et al*., 2019a; Zaharia *et al*., 2019b).

Sensory analysis evaluation. It represents one of the most important analysis of oil-based emulsions used for appreciation and compare of its sensory properties, *i.e.* adhesion, degree of emulsifying, in-time stability, consistency and odor/smell. There were performed tastings by a competent commission of five members (a panel of 5 trained judges). Each sensory property was appreciated in a score range of 1 - 5 points (*i.e.* 1 - extremely poor, 2 - poor, 3 - satisfactory, 4 - good, and 5 - excellent), during 1 till 6 days of storage period, after the method proposed by Banu (Banu, 2002; Dănilă *et al*., 2019; Zaharia *et*

al., 2018; Zaharia *et al*., 2019a; Zaharia *et al*., 2019b). After the statistical processing of all scores, it was appreciated the prepared *iR* emulsion quality.

The overall appreciation of the prepared emulsion samples was measured on the same scale and referred to as overall quality.

2.3. O/W Emulsion Preparation Methodology

The emulsions containing beeswax as carrier matrix and rosemary essential oil (*iR*) were formulated and prepared.

Beeswax is a slow, biodegradable and viable valuable absorbent. It is used for creation of a covering onto materials with bioactive ingredients under the form of a hydrophobic layer.

The emulsions were prepared in three distinct steps: (*i*) beeswax was melted at 65°C in a thermostatic water bath (700 rpm); (*ii*) glycerin, Tween solution (30%) and water were added in the melted beeswax, and (*iii*) after cooling to 40°C of the mixture, the rosemary essential oil (i*R*) was added in the dropwise under continuous stirring.

In the composition of emulsions was varied the amount of essential oil and beeswax, the other measures being kept constant as in Table 2.

Table 2
Formulation of Rosemary Essential Oil-Beeswax-Based Emulsions

Emulsion	1*R*	2*R*	3*R*	4*R*	5*R*	6*R*	7*R*	8*R*	9*R*
Beeswax, [g]	0.06	0.36	0.06	0.36	0	0.42	0.21	0.21	0.21
Glycerin, [mL]	1	1	1	1	1	1	1	1	1
Water, [mL]	6.82	6.52	6.22	5.92	6.58	6.16	6.79	5.95	6.37
Tween 80, [mL]	2	2	2	2	2	2	2	2	2
Rosemary oil, [mL]	0.12	0.12	0.72	0.72	0.42	0.42	0	0.84	0.42

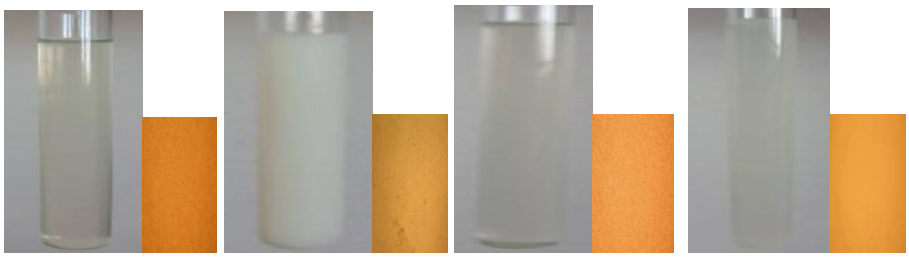
3. Results and Discussion

There are necessary serious investigations for selection of a few emulsion compositions with *increased value* and *physical*, *oxidative* and *microbiological stability*.

From the nine prepared emulsions, stable and adequate for use in added-value textiles manufacturing were found to be only four emulsions, *i.e.* 3*R*, 4*R*, 8*R* and 9*R*.

3.1. Characteristics and 'In-Time' Stability of Selected *iR* Emulsions

The visual images for the four selected emulsions based on rosemary essential oil and beeswax were illustrated in Fig. 1.



3*R* (*a*) 4*R* (*b*) 8*R* (*c*) 9*R* (*d*)

Fig. 1 – Emulsions (*iR*) appearance.

Related to visual images, the 4R and 8R emulsions are homogeneous, white, without agglomerations of particles and easily handle, but emulsions 3R and 9R are translucent and free of particles in suspension. The dispersed phase is represented as a compact, dense small globular mass (according to microscopic images illustrated in Fig. 1).

Emulsion stability and quality can be affected in period of storage due to possible alteration processes, based on slow oxidation and conversion in a complex organic system, a little lower homogeneous and with a relative high number of new components formed in different steps of alteration process (*i.e.* initiation, development and breaking of different macromolecular chains).

The dynamic of primary oxidation products (*i.e.* hydroperoxides) formation in the period of emulsions storage is very important and must be known. That is why it must be periodically analyzed the acidity indice (*AI*), peroxide indice (*PI*), total contents of conjugated dienes (*CD*) and trienes (*CT*), as well as the content of other categories of majority or minority constituents (*e.g.*, the evolution in time of the total content of polyphenols for demonstration of antibacterial action added to impregnated textile material, as well as the study of evolution of total flavonoids content for validation of resistance and stability of textile material in time, and also of initiation of some oxidative or reductive alteration processes through monitoring of the variation in case of the value variation of acidity index, peroxide index, etc.).

Physical-chemical quality indicators of the *iR* selected emulsions for textile impregnation testing, *i.e. 3R, 4R, 8R,* and *9R,* were varied in-time according with data from Table 3, in range of 30.54-48.65% for total fatty matter separated (FM), 51.35-69.46% for aqueous phase, 5.5-5.6 (±0,3) for pH, 1.024-1.106 g/cm^3 for absolute density normalized at 20°C, 1.1919-3.3195 mg KOH/g of emulsion for acidity indice (AI), 3.5696-15.2555 mmol/g of emulsion for peroxide indice (PI), 1.0099-13.4432 mmol/g of emulsion for total content of conjugated dienes (CD), 0.7612-0.9643 mmol/g of emulsion for total content of conjugated trienes (CT), 0.4157-0.9836 μg/g of emulsion for total content of polyphenols (TPF) and 0.5433-1.7694 μg/g of emulsion for total content of

flavonoids (TF). All these characteristic values are corresponding and respect the recommended norms for skin care (cosmetics) and pharmacy products.

Table 3
The Values of Some Physical-Chemical Quality Properties and Indicators for Selected Emulsions Based on Beeswax-Essential Rosemary Oil

Physical-chemical quality indicators	Storage time	Values for *iR* prepared emulsions			
		3*R*	4*R*	8*R*	9*R*
pH (room temperature, t=23.7°C)	initial	5.7	5.6	5.6	5.6
	1 month	5.7	5.6	5.6	5.6
	8 months	5.6	5.5	5.5	5.5
Density (absolute) (20°C), [g/cm^3]	initial	1.028	1.024	1.026	1.028
	1 month	1.024	1.020	1.022	1.025
	8 months	1.020	0.988	1.002	0.984
Density normalized (20°C), [g/cm^3]	initial	1.031	1.027	1.029	1.031
	1 month	1.028	1.024	1.026	1.028
	8 months	1.106	1.078	1.080	1.048
Acidity indice (AI), [mg KOH/g of emulsion]	initial	1.1919	1.7534	1.6625	1.2752
	1 month	1.2958	1.8282	1.7884	1.2812
	8 months	3.3195	2.3337	2.3943	1.6625
Peroxide indice (PI), [mmol/g of emulsion]	initial	4.0547	4.6272	3.5696	4.0650
	1 month	4.8648	4.6496	4.6842	5.9644
	8 months	15.2555	4.8990	10.5485	13.1666
Total content of conjugated dienes, [μmol/g of emulsion]	Initial	1.0099	12.8089	4.2693	1.4713
	1 month	1.0112	12.9443	4.2740	1.5048
	8 months	1.6432	13.4432	4.3112	1.9863
Total content of conjugated trienes, [μmol/g of emulsion]	Initial	7.8784	11.7612	3.5141	1.0514
	1 month	7.6452	11.4695	3.5002	1.0446
	8 months	6.6426	10.1032	3.3868	0.9643
Total content of polyphenols (TPF), [μg/g of emulsion]	Initial	0.4157	0.9836	0.4413	0.4299
	1 month	0.4652	0.8946	0.4985	0.4752
	8 months	0.5516	0.7560	0.6419	0.5929
Total content of flavonoids (TF), [μg/g of emulsion]	Initial	0.5433	1.7365	0.8090	0.6076
	1 month	0.5522	1.7398	0.8112	0.6101
	8 months	0.5986	1.7694	0.8213	0.6226

A few properties were modified due to separation of aqueous and fatty phases and also different alterations caused of organics degradation in-time (easy oxidation, reduction, or/and adsorption). Overall, the selected emulsions had a relative good stability till 1-3 months, and adequate sensory quality. Moreover, the prepared emulsions had contained relative satisfactory contents of total polyphenols and flavonoids, fact which can suggest that these emulsions can have beneficial antioxidant action due to their potential antibacterial activity against active microorganisms in contact with.

The peroxides and hydroperoxides are instable compounds, and in period of emulsions storage (stocking) these can be decomposed with formation of secondary oxidation products such as aldehydes, cetones and its derivatives with carbonyl chains of different lengths (Dănilă *et al*., 2019). Peroxides did not have a direct influence against the sensory properties of emulsions, and if aldehydes and cetones are formed, these have specific odor (usually a rancid smell). The experimental results indicated that the odor of prepared emulsions was not significantly modified for a storage period of 9-10 months and no rancid smell appeared. As result, during the storage period the emulsions were not supposed to major destructive actions, only easy superficial primary oxidative and/or reductive modifications through increasing of the values of acidity and peroxide indexes, content of conjugated dienes and trienes (for a few emulsions, the content of conjugated trienes decreased), and the secondary oxidation products of cetone and aldehyde types were not formed, fact evidentiated by keeping the same odors of prepared emulsions, a little bit weaker not so intense as at the beginning.

3.2. Sensory Analysis Evaluation

Chemical composition of each *iR* emulsion influences the sensory properties of final product, *i.e.* adherence, consistency and odor are dependent of characteristics of emulsion constituents and also its complex physical-chemical and biological alterations which can occur in.

In general, the sensory properties are appreciated as result of testing. A commission with five specialists was evaluated organoleptically the selected emulsions with the main scope of appreciation and comparison the emulsion sensory properties, *i.e.* adherence, degree of emulsifying, stability in time, consistence and odor. Each specialist completed an individual report with scores which can vary from 1 till 5.

The synthesis of prepared (*iR*) emulsions sensory analysis is illustrated in Fig. 2.

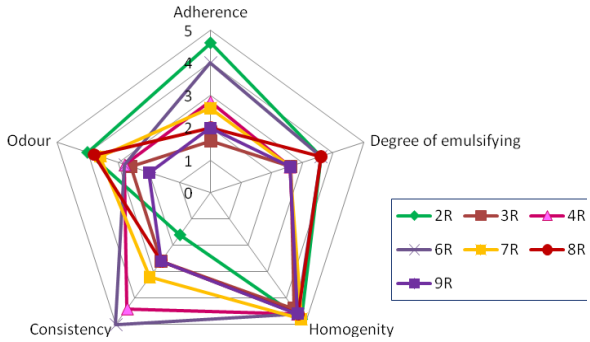


Fig. 2 – Diagram of sensory analysis for rosemary oil-beeswax-based emulsions (*iR*).

After statistical data processing, it was appreciated the quality of prepared emulsions for validation of the most indicated emulsion to be used for impregnation of textiles for its added value (*e.g.* antibacterial activity) (Radu *et al*., 2017). Moreover, it was concluded that the most recommended rosemary essential oil-beeswax-based emulsion for textile fabric impregnation (cotton, or viscose) is the 4*R* emulsion, followed by 8*R* emulsion, reported to the Romanian textile manufacturer.

4. Conclusions

A series of beeswax-rosemary essential oil-based emulsions (*iR*, *i*=1…9) were prepared for use to impregnate a few textile materials by a Romanian textile manufacturer in order to add mainly anti-bacterial value to the selected textiles used in aromatherapy and skin care benefits. The physical-chemical quality indicators of investigated *iR* emulsions based on rosemary essential vegetal oil-beeswax matrix were indicated the highest stability of tested prepared emulsions.

The comparative analysis of experimental results performed for the acidity index (*AI*) and peroxide index (*PI*) were permitted a few preliminary information concerning the dynamic of *iR* emulsion behaviour during the storage activity at room temperature (1 and more than 8 months). The results indicated the separation of aqueous and organic phases, the formation of a few primary oxidation products (hydroperoxides), but not formation of secondary oxidation products (aldehydes and ketones) with their specific rancid odor.

The general physical-chemical indicators of investigated *iR* emulsions were found corresponding with the standardized norms in cosmetics/foods, pharmacy and textiles for applications in added-value textiles manufacturing for its antibacterial action (satisfactory content of polyphenols and flavonoids).

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EMULSII PE BAZĂ DE SISTEM ULEI DE ROZMARIN – CEARĂ DE ALBINE FOLOSITE PENTRU FABRICAREA DE MATERIALE TEXTILE CU VALOARE ADĂUGATĂ PENTRU BENEFICII DE ÎNGRIJIRE A PIELII

(Rezumat)

Această lucrare prezintă pe scurt metodologia de preparare a nouă emulsii pe bază de sistem matrice ceară de albine – ulei esențial de rozmarin (*i**R**, i*=1…9) și caracteristicile lor specifice de calitate, reprezentative fiind emulsiile 3*R*, 4*R*, 8*R* și 9*R* pentru fabricarea de materiale textile cu valoare adăugată pentru aromaterapie și

beneficii de îngrijire a pielii. Aceste emulsii preparate au fost preliminar caracterizate prin câteva proprietăți specifice fizico-chimice și indicatori de calitate (*i.e*. pH, densitate absolută, indice de aciditate, indice de peroxid, conținut total de diene și triene conjugate, conținut total de polifenoli și flavonoide) precum și analiză senzorială care a permis recomandarea celei mai indicate emulsii pentru utilizare de către producătorul textil român pentru adăugare de valoare produselor sale textile, considerând stabilitatea emulsiilor în timp (după 1 și 8 luni de depozitare la temperatura camerei) și posibila acțiune antibacteriană după impregnarea materialului textil.

Această lucrare subliniază de asemenea că cea mai recomandată emulsie este 4*R* urmată de 8*R*, ambele având o stabilitate relativ bună în timp până la separarea fazelor organică și apoasă, precum și un conținut suficient de polifenoli și flavonoide.