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Evaluation of tobacco seed oil as a sustainable ingredient in oil-in-water (O/W) emulsion creams

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SUMMARY: Tobacco seeds, which are abundant and rich in oil, offer a sustainable alternative for cosmetic applications due to their fatty acid profile, which is similar to grape seed oil. This study compares tobacco seed oil (Krumovgrad 58 variety, Bulgaria) with commercial grape seed oil for use in cosmetic emulsions. Fatty acid composition and tocopherol content were analyzed, and emulsions were evaluated for physicochemical properties (peroxide value, acid value, refractive index, oxidative stability, and pH) and microbiological quality. The results showed that the emulsions had a moisture content of 82.2–85.1%, refractive index of 1.624–1.675, and oxidative stability of 2.60–3.85 hours. A microbiological analysis revealed fewer than 10 cfu/g of microorganisms, including molds and yeasts, in all emulsions. The findings demonstrate that tobacco seed oil exhibits comparable properties to grape seed oil, making it a viable, sustainable option for cosmetic formulations.

KEY-WORDS: *Cosmetic; Emulsion; Moisturizin; Tobacco seed oil*

RESUMEN: ***Evaluación del aceite de semilla de tabaco como ingrediente sostenible en cremas de emulsiones de aceite en agua (O/W).*** Las semillas de tabaco, abundantes y ricas en aceite, ofrecen una alternativa sostenible para aplicaciones cosméticas debido a su perfil de ácidos grasos, similar al del aceite de semilla de uva. Este estudio compara el aceite de semilla de tabaco (variedad Krumovgrad 58, Bulgaria) con el aceite de semilla de uva comercial para su uso en emulsiones cosméticas. Se analizó la composición de ácidos grasos y el contenido de tocoferoles, y se evaluaron las emulsiones en cuanto a sus propiedades fisicoquímicas (índice de peróxidos, índice de acidez, índice de refracción, estabilidad oxidativa, pH) y calidad microbiológica. Los resultados mostraron que las emulsiones tenían un contenido de humedad del 82,2–85,1 %, un índice de refracción de 1,624–1,675 y una estabilidad oxidativa de 2,6–3,85 horas. El análisis microbiológico reveló menos de 10 ufc/g de microorganismos, incluidos mohos y levaduras, en todas las emulsiones. Los hallazgos demuestran que el aceite de semilla de tabaco presenta propiedades comparables a las del aceite de semilla de uva, lo que lo convierte en una opción viable y sostenible para formulaciones cosméticas.

PALABRAS CLAVE: *Aceite de semilla de tabaco; Cosmética; Emulsión; Hidratante.*

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1. INTRODUCTION

Tobacco seed oil is derived from the seeds of *Nicotiana tabacum* L., a byproduct of tobacco plant cultivation. *Nicotiana tabacum* L., commonly known as cultivated tobacco, is a plant belonging to the Solanaceae family. It is grown worldwide, with Bulgaria being one of the leading producers within the European Union. Among the distinguished tobacco varieties, those from the Basma group, particularly the Bulgarian cultivars Krumovgrad 90 and 58, hold considerable importance in both cultivation and industrial applications. These tobacco varieties have been cultivated using both conventional agricultural practices and organic farming methods.

Tobacco seeds are in large quantities per plant, which makes them a good source for obtaining oil. Tobacco seed oil is indicated for many applications, but mostly for diesel fuel (Grisan *et al*., 2016). It is also known as suitable for use in cosmetic products and the pharmaceutical industry. The application of tobacco seed oil across various industries is driven by the high oil content of tobacco seeds (30–50%) and their fatty acid (FA) profile, particularly the significant presence of polyunsaturated fatty acids (PUFAs) (Ashirov *et al*., 2020). Linoleic acid, the most abundant PUFA in tobacco seed oil, constitutes 65–75% of the total FA content. Linoleic acid is not synthesized in the body and it is a precursor for metabolic reactions and the formation of prostaglandins, which can regulate the inflammatory response of the skin (Srbinoska *et al*., 2012). In recent research, tobacco oil is reported to have anti-inflammatory activities by inhibiting the production of pro-inflammatory cytokines and whitening activity by inhibiting tyrosinase activity and cellular melanin production (Gu *et al*., 2022). Tobacco seed oil is a good source of natural antioxidants as its tocopherol content constitutes all tocopherol derivatives α-, β-, γ- and δ-, as reported by Zlatanov *et al*, (2007) in tobacco seeds from different varieties.

On the other hand, tobacco oil has similar fatty acid content, regarding the individual presence of linoleic acid and tocopherol content, to one of the most common oils in the cosmetic industry – grape seed oil (Popova *et al*, 2018). Grape seed oil, a carrier oil (fixed oil), is often used to dilute essential oils before topical application to prevent skin irritation (Marshall *et al*, 2023). Grape seed oil has a high PUFA content (85 – 90%), and linoleic acid contributes between 66.0 and 75.3% of total FA. Grape seed oil is used as an emollient in cosmetic emulsions and facial cream formulas with an anti-aging effect (Garavaglia *et al*., 2016). This brief review of the use of grapeseed oil for embedding in emulsions gives a reason to suggest a possible similar application for tobacco seed oil.

The emulsions are commonly composed of two basic ingredients – oil and water and can be classified as oil-in-water emulsions (O/W), water-in-oil emulsions (W/O), and multiple emulsions (W/O/W) (O/W/O). The formula of emulsion also contains active ingredients such as emollients, humectants, thickeners, emulsifiers, stabilizers, preservatives, and neutralizers (Calvo *et al*., 2020). The oil – in - water (O/W) emulsion is preferred by consumers despite the large volume of water in its formulation, which serves as a perfect growth factor for bacteria. To prevent bacteria from growing, essential oils could be used as natural preservatives in the emulsion preparation. Essential oils, properly selected and used at a concentration of 0.5–2.5%, demonstrate a high antimicrobial potential. Tea tree (*Melaleuca alternifolia*), thyme (*Thymus vulgaris*), lemongrass (*Cymbopogon citratus*), oregano (*Origanum vulgare*), rosemary (*Rosmarinus officinalis*), calamint (*Calamintha officinalis*), or lavender (Lavendula officinalis) have received a lot of attention recently because they have antimicrobial properties and have been suggested as natural preservatives. (Shkreli *et al*., 2022). Lemongrass essential oil, in particular, contains various compounds, such as citral, geraniol, and citronellal, which contribute to its aroma and flavor, as well as its potential interactions with other ingredients. Lemongrass essential oil contains compounds with antioxidant properties that could potentially affect the oxidative stability of the emulsion. While the antioxidant compounds in lemongrass essential oil can help protect oils from oxidation, interactions with other antioxidants or prooxidants in the emulsion formula can affect overall oxidative stability.

This study aims to explore the potential use of tobacco seed oil in oil-in-water (O/W) emulsions and evaluate its suitability as a substitute for grape seed oil in the formulation of emulsion-based creams with natural preservative – lemongrass essential oil.

The following part of this paper moves on to describe in detail the physicochemical parameters, the chemical composition of tobacco seed oil, the formula of the emulsion, and its antimicrobial activity. The focus of the research is directed towards comparing the results obtained for an emulsion cream containing tobacco seed oil and grape seed oil.

2. MATERIALS AND METHODS

2.1. Materials

Tobacco seeds from organically grown tobacco variety Krumoovgrad 58 were collected from the Tobacco and Tobacco Products Institute, part of Agriculture Academy, Bulgaria. The tobacco plants were grown in the field at the “Experimental Tobacco Station” Department – Gotse Delchev.

Refined grape seed oil (Mapa International LTD, Spain) purchased from the commercial network was used. *Cymbopogon flexuosus* leaf oil (lemongrass oil) was purchased from Bioherba R Ltd (produced in EU- 4102 Bulgaria). All the following ingredients used for the cosmetic emulsion formulation: glycerin, glyceryl stearate (SE), cetearyl alcohol, beeswax, and dimethicone, were analytical degree, purchased from Sigma Aldrich, Germany.

2.2. Methods

2.2.1. Oil extraction

Tobacco oil was obtained by solvent extraction with n-hexane using a Soxhlet apparatus. The extraction was conducted according to the ISO 659:2014 for 6-8 h. After extraction, the solvent was evaporated on a rotary vacuum evaporator.

2.2.2. Emulsion preparation

An oil-in-water (O/W) emulsion base was prepared with both tobacco seed oil (E1) and refined grape seed oil (E2). The emulsion was set in two phases – A and B. The A phase consisted of 2.0 g glycerin and 98.0 g distilled water. The B phase contained 6.0 g glyceryl stearate, 1.5 g cetearyl alcohol, 1.0 g beeswax, 9.0 g tobacco or grape seed oil, and 1.0 g dimethicone. All the components were heated up to 70–75 ºC until the components from phase B melted. The emulsification was carried out by adding phase B into phase A, with continuous stirring on a Tekmar Tissumizer SDT-1810 Homogenizer TR-10. The emulsion was cooled to 40 ºC during stirring. *Cymbopogon flexuosus* leaf oil (lemongrass essential oil) was incorporated into the emulsions at a concentration of 0.5%. The four obtained emulsions (E1 – with tobacco seed oil; E1-LO – with tobacco seed oil and 0.5% lemongrass essential oil; E2 – with grape seed oil; E2-LO – with grape seed oil and 0.5% lemongrass essential oil) were prepared for analysis.

2.2.3. Peroxide value (POV)

The method involved a titration of the oil with 0.002N $Na_2S_2O_3$. 0.1 – 0.2 g of the tobacco or grape seed oil were transferred into a flask and 5 mL of $CHCl_3$, 2.5 mL of CH_3COOH, and freshly prepared 50% KI (1 mL) were added. After 5 min in the dark, 10 – 20 mL H_2O and 1% starch (1 mL) were added to the solution and a slightly purple color was obtained. The titration with 0.002N $Na_2S_2O_3$ continued until a slight yellow or colorless endpoint. Blank titration under the same conditions must be carried out (ISO 3960:2007). The following formula was used to calculate the POV:

POV= ((V-V”0”) x 0.002 x 1000)/m, meqO_2/kg

where: V – volume of 0.002N $Na_2S_2O_3$ used for titration of the oil sample, mL

V”0” – volume of 0.002N $Na_2S_2O_3$ in the blank test, mL

0.002 N – normality of $Na_2S_2O_3$

m – weighed portion of oil, g

2.2.4. Acid value (AV)

The acid value is expressed as the amount of potassium hydroxide (KOH) required to neutralize the free fatty acids in one gram of oil. According to ISO 660:2009, 1-5 g of tobacco or grape seed oil were measured and transferred into the flask. An alcohol-ether solution (1:2, v/v) and 1% phenolphthalein were mixed and neutralized with KOH. A pre-neutralized solution of 50 mL was transferred into the oil flask and titration with 0.1N alcoholic KOH was performed until a pink color appeared and was constant for 30 s. The acid value was calculated:

AV=VxFx5.611/m, mg KOH/g

where: V – volume of 0.1N KOH, used for titration of the oil sample, mL

F – factor of 0.1N KOH

m – weighed portion of oil, g

5.611 – correction factor/volume of KOH in mg, for 1 mL 0.1N KOH

2.2.5. Oxidative stability

The oxidative stability of the oils and emulsions was performed with Rancimat 679 (Metrohm, Switzerland). The method is based on the conductometrical measurement of the volatile compound which is formed from lipid oxidation when the air of 20 dm^3/h passes through the sample and the temperature is 100 ºC for the glyceride oils and 110 ºC for the emulsions. The conductivity is continuously recorded and the induction time is graphically presented. The induction time is the indicator of resistance to oxidation of the lipids (ISO 6886:2006).

2.2.6. Refractive index (RI)

The refractive index represents the ratio of the speed of light in a vacuum to the speed of light in the given medium. It is measured by refractometry at 25 ºC with a refractometer of ABBE, HPB002 Zuzi/AUXILAB, with a ratio 1.3000–1.7000 and 0.005 accuracy (BDS EN ISO 6320:2000).

2.2.7. pH value

The pH of the emulsion was determined using a pH meter AD12 Adwa, Hungary. The measurement was performed immediately after the emulsification and cooling of the emulsions, after 48 and 90 days, stored in a refrigerator.

2.2.8. Fatty acid composition

The determination of the fatty acid composition of the oils was made using the GC method. The oil was subjected to pre-esterification with methanol in the presence of sulfuric acid. The obtained fatty acid methyl esters (FAMEs) were subjected to an Agilent 8860 gas chromatograph. A DB–Fast FAME column (30 m x 0.25 mm x 0.25 μm (film thickness)) and flame ionization detector (FID) were used. The injector and detector temperatures were set at 270 and 300 °C. The column temperature was increased from 70 °C (1 min), at 6 °C/min to 180 °C (0 min), and at 5 °C/min to 250 °C, and the split ratio was 50:1. A standard Supelco, USA mixture (FAME mix 37 components, Supelco, USA) was used for the identification of FAMEs (ISO 12966-1:2014; ISO 12966-2:2011).

2.2.9. Tocopherol content

Individual tocopherols were determined by Merck-Hitachi (Merck, Darmstadt, Germany) high-performance liquid chromatography (HPLC) with fluorescence detection (excitation at 295 nm and emission at 330 nm) and Nucleosil Si 50-5 (250 mm x 4 mm) column. n-Hexane:dioxane, 96:4 (v/v) was used as the mobile phase and the flow rate was set at 1 mL/min (ISO 9936:2016).

2.2.10. Detection of microorganisms

The detection of microorganisms was made by the diffusion method in agar well. Standard methods were used to enumerate and detect aerobic mesophilic bacteria, yeast, and mold (ISO 1149:2017; ISO 16212:2017). The samples were also tested for Gram-positive (*Candida albicans* – ISO 18416:2015, *Staphylococcus aureus* – ISO 22718:2015) and Gram-negative bacteria (*Escherichia coli* – ISO 21150:2015, *Pseudomonas aeruginosa* – ISO 22717:2015).

2.3. Statistical analysis

All analyses were performed in triplicate. The results are presented as mean values ± standard deviation. Microsoft Excel Office was used for data calculations.

3. RESULTS AND DISCUSSION

Plant oils are used in cosmetic formulations as natural emollients. They are known as fixed or carrier oils which provide health benefits for human skin (Alvarez and Rodriguez, 2000). To assess the potential of tobacco seed oil as a carrier oil, two approaches were employed: (1) analysis of tobacco seed oil and cosmetic emulsions formulated with it, and (2) analysis and comparison with a well-established carrier oil—grape seed oil. Grape seed oil was selected as a reference due to its similar fatty acid composition to tobacco seed oil, as documented in the literature (Ashirov et al., 2020; Hussein and Abdrabbla, 2015).

3.1. Analyses of tobacco and grape oils

To consider the quality of tobacco seed oil for cosmetic use, some main physical parameters were analyzed and are shown in Table 1.

The tobacco seed oil had a lower peroxide value than the grape seed oil. The peroxide value for tobacco oil (2.15±0.37 meqO_2/kg) was lower than the reported values for other cosmetic oils such as argan oil (3.2±1.0 meqO_2/kg) (Gharby *et al.*, 2013). The tobacco seed oil, with a lower peroxide value in the range of 1–3 meqO_2/kg, could be preferred for cosmetic products.

The acid value for the tobacco seed oil was significantly higher 3.26±0.04 mg KOH/g than the result obtained for grape seed oil at 0.18±0.01mg KOH/g. The acid value represents the quantity of free fatty acids in the oil. Tobacco oil is expected to have greater percentage of free fatty acids compared to that of grape seed oil since it has not been refined. Despite its higher acid value, tobacco oil falls within the limits of the recommended value of lower than 4.0 mg KOH/g oil. Bialek *et al.,* (2016) reported unconventional plant oils for cosmetic use with acid values in the range of 0.17 – 3.96 mg KOH/g oil.

Both oils have a refractive index of over 1.470. The refractive index (RI) represents the reflection of light between two mediums. This factor is important for cosmetic products because it could define the field of application, as the human skin has an RI between 1.30 and 1.55 (Gajinov *et al.*, 2010). The tobacco and grape seed oils had almost the same RI (Table 1), higher than the reported edible oils index in Codex Alimentarius (1.448 – 1.451) but in the range of skin RI.

The iodine value was calculated for tobacco and grape seed oil. The iodine value of both oils was close, which can be explained by their fatty acid composition (Table 3).

The oils had good oxidative stability with great induction time for the tobacco oil- stable for 12.20±0.10 h and the grape oil for 9.20±0.10 h. The slower oxidation of the tobacco seed oil was proved with its oxidative stability for 12.2 h at 100 ºC. The grape seed oil was reported by Maszewska *et al.*, (2018) with a 2.4 h induction time, which was very low in comparison to the induction time for tested grape oil (Table 1). Tobacco oil had similar induction time in comparison with oils from unconventional raw materials such as parsley, tomato, and basil (Symoniuk *et al.*, 2022).

TABLE 1. Physical parameters of tobacco and grape seed oils

Physical parameters	Tobacco seed oil	Grape seed oil
Peroxide value, meqO_2/kg	2.15±0.37	3.45±0.07
Acid value, mg KOH/g	3.26±0.04	0.18±0.01
Iodine value, g I_2/100g	136.85±0.20	134.58±0.24
Refractive index	1.476±0.000	1.473±0.000
Oxidative stability (100 °C), h	12.20±0.10	9.20±0.10

*Mean ± SD, n=3

Oil derived from *Cucurbita pepo*, characterized by a refractive index of 1.4695, an acid value of 2.32 mg/g, an iodine value of 148 mg/100g, and a free fatty acid content of 22.50 mg/g, was utilized in the formulation of an emulsion cream, reported by Pratibha Gavarkar *et al*., (2016) suitable for use in cosmetic products. However, the resulting cream exhibited a higher microbial count compared to emulsions formulated with tobacco seed oil.

3.1.1. Tocopherol content in oil

The tocopherol content in the oils was presented by three tocopherols: α-, γ-, and δ- and two tocotrienols: α- and γ-. The total content of vitamin E in tobacco seed oil was 175.0±2.1 mg/kg, almost half the content in grape seed oil 498.0±4.2 mg/kg. δ- Tocopherol predominates in tobacco seed oil (68.4±0.6%); while in grape seed oil this tocopherol was not detected. The grape seed oil was rich in α- tocopherol, 52.4±1.7%. The group of tocotrienols – α- tocotrienol and γ- tocotrienol are presented only in the grape seed oil. The quantity of γ- tocopherol in both oils showed a similar value (Table 2). The tocopherol analysis of both oils could define them as rich in vitamin E. The high level of vitamin E is a good supplement in cosmetics, as tocopherols are natural antioxidants and protect polyunsaturated fatty acids from free radical-induced oxidation (Alvarez and Rodriguez, 2000). The high level of tocopherol content in grape oil with a focus on α- tocopherol content implies greater stability of the oil in terms of oxidative stability. On the other hand, α- tocopherol could have pro-oxidant activity in plant oils depending of the concentration.

TABLE 2. Tocopherol contents in tobacco and grape seed oils

Tocopherols	Tobacco seed oil	Grape seed oil
α- tocopherol, %	7.1±0.1	52.4±1.7
α- tocotrienol, %	N/D*	9.2±0.4
γ- tocopherol, %	24.5±1.1	25.2±0.6
γ- tocotrienol, %	N/D	13.2±0.8
δ- tocopherol, %	68.4±0.6	N/D
Total, mg/kg	175.0±2.1	498.0±4.2

*N/D – not detected
*Mean ± SD, n=3

3.1.2. Fatty acid composition of oils

The fatty acid composition of tobacco seed oil and grape seed oil used for the emulsions was analyzed and is presented in Table 3. Both oils are polyunsaturated, with a high presence of linoleic acid ($C_{18:2}$) – 65.90±0.80% for tobacco seed oil and 60.16±0.20% for grape seed oil. This n-6 fatty acid is essential for humans and plays a role in the formation of ceramides in skin layers. The monounsaturated fatty acid content was 18.50±0.25% in tobacco seed oil and 27.55 ±0.25% in grape seed oil (Figure 1). Oleic acid ($C_{18:1}$) was the most abundant monounsaturated fatty acid, with its content in grape seed oil being 10.0% higher than in tobacco seed oil. Palmitic acid ($C_{16:0}$) was the predominant saturated fatty acid in both oils. A small amount of linolenic acid ($C_{18:3}$) was detected in both oils, with the n-6 isomer present in both and the n-3 isomer found only in grape seed oil. The ratio of saturated (SFA), monounsaturated (MUFA), and polyunsaturated (PUFA) fatty acids in tobacco seed oil was 1:1:4, while in grape seed oil, it was 1:2:4.

TABLE 3. Fatty acids in tobacco and grape seed oils

Fatty acids, %		Tobacco seed oil	Grape seed oil
$C_{8:0}$	Caprylic	N/D*	0.04±0.01
$C_{14:0}$	Myristic	0.10±0.00	0.04±0.01
$C_{16:0}$	Palmitic	12.00±0.50	7.98±0.21
$C_{16:1}$	Palmitoleic	0.20±0.05	0.12±0.01
$C_{17:0}$	Margarinic	0.20±0.03	0.07±0.02
$C_{17:1}$	Heptadecanoic	0.40±0.05	0.05±0.01
$C_{18:0}$	Stearic	2.10±0.01	3.14±0.55
$C_{18:1}$	Oleic	17.70±0.20	27.11±0.30
$C_{18:2}$	Linoleic	65.90±0.80	60.16±0.20
$C_{18:3\ n\text{-}6}$	Linolenic	0.60±0.20	0.04±0.02
$C_{18:3\ n\text{-}3}$	Linolenic	N/D	0.43±0.03
$C_{20:0}$	Arachidic	0.20±0.05	0.25±0.05
$C_{20:1}$	Eicosenoic	0.10±0.04	0.24±0.05
$C_{22:0}$	Behenic	0.40±0.05	0.30±0.05
$C_{22:1}$	Erucic	0.10±0.00	0.03±0.00

*N/D – not *detected*
**Mean ± SD*, n=3

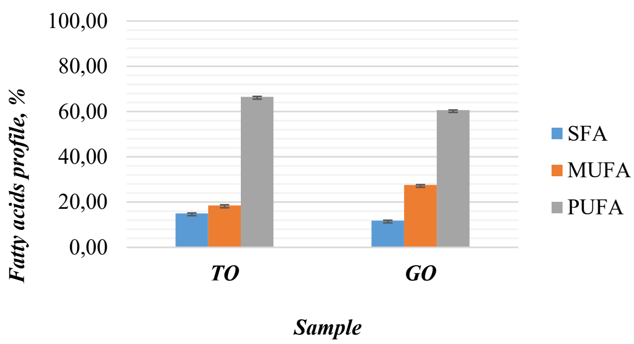


FIGURE 1. Fatty acid composition of TO - tobacco oil, GO - grape oil, %. TO – tobacco oil; GO – grape oil; SFA – saturated fatty acids; MUFA – monounsaturated fatty acids; PUFA – polyunsaturated fatty acids
*Mean ± SD, n=3

The human epidermis contains fatty acids in its structure, such as linoleic acid (21.5%), oleic acid (15.1%), palmitic acid (14.0%), stearic acid (11.1%), and lignoceric acid (10.0%). These fatty acids play a transport function in the skin layers, with linoleic acid being particularly crucial for maintaining the strength of the skin's water permeability barrier (Kunik *et al.*, 2022). The fatty acid composition of tobacco seed oil and grape seed oil aligns with the reported literature and is considered relevant (Ashirov *et al*., 2020; Hussein and Abdrabbla, 2015). The ideal lipid has a ratio of saturated (SFA), monounsaturated (MUFA), and polyunsaturated (PUFA) fatty acids of 1:1:1. The fatty acid distribution of the examined oils (1:1:4 for tobacco seed oil and 1:2:4 for grape seed oil) was close to that of the ideal lipid, as both oils exhibited higher levels of PUFA due to their high linoleic acid contents (Selwyn and Govindaraj, 2023). Tobacco seed oil cold be considered closer to the ideal lipid fatty acid distribution compared to grape seed oil.

3.2. Analysis of the emulsions

The tobacco and grape seed oils were used for preparing emulsion creams – base emulsion creams (E1 and E2) and creams with added lemongrass essential oil (E1 - LO and E2 - LO). The Natural Products Association (NPA) and the Organic and Sustainable Industry Standards (OASIS) define a product as 'natural' when it is composed of at least 95% ingredients which are derived from natural sources. The NPA has previously established a list of substances considered safe for such use – the

NPA Illustrative List. The ingredients for the emulsion were selected with an emphasis on organic and natural components whenever possible. Water and glycerin were used as humectants, glyceryl stearate SE as an emulsifier, and beeswax as a co-emulsifier. To adjust the viscosity, cetearyl alcohol was incorporated. Tobacco seed oil, grape seed oil, and dimethicone were included as emollients. The dimethicone is considered an organic/inorganic polymer with an anti-foaming effect and a protective function (Mancuso *et al.*, 2022).

O/W emulsion was chosen because of its capacity to reduce greasiness and its content of emollients can be between 5 and 30% (Chao *et al*., 2018). The base emulsion creams are shown in Figure 2 (a, b) immediately after preparation. They are homogenous with white color without lumps, without any smell, and completely washed after dilution. The lemongrass essential oil was added to the prepared base emulsion creams after cooling, imparting a pleasant citrus aroma to the emulsion.

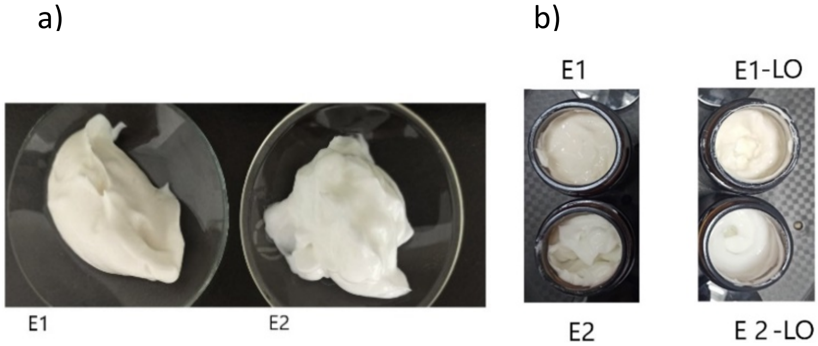


FIGURE 2. a) Emulsion cream bases with tobacco oil - E1 and with grape oil - E2
b) Emulsion bases and bases with lemongrass essential oil
E1 – tobacco oil emulsion; E1-LO – tobacco oil emulsion with 0.5% lemongrass oil;
E2 – grape oil emulsion; E2-LO – grape oil emulsion with 0.5% lemongrass oil

Some physical parameters for all the emulsions were provided in the study (Table 4).

TABLE 4. Physical parameters of emulsions with tobacco and grape seed oils

Emulsion cream	**E1**	**E1 - LO**	**E2**	**E2 - LO**
Emollient / ***Physical parameters***	**With tobacco seed oil**	**With tobacco seed oil and 0.5% lemongrass essential oil**	**With grape seed oil**	**With grape seed oil and 0.5% lemongrass essential oil**
Moisture, %	82.2±0.8	81.1±0.5	85.1±0.7	84.0±0.7
Refractive index	1.644±0.000	1.651±0.000	1.624±0.000	1.675±0.000
Oxidative stability (110 ºC), h	2.60±0.01	3.85±0.02	2.77±0.01	2.68±0.02

*Mean ± SD, n=3

The moisture content in all emulsions exceeded 80.0%. The emulsions containing tobacco oil E1 and E1 - LO had lower pH values than those with grape seed oil. The pH value measured immediately after preparation indicated an alkaline nature for all of the emulsions, with pH >7 (Figure 3). After 48 days, the pH of the emulsions decreased. The E1 emulsion (with tobacco seed oil) had pH 6.64, which remained constant for up to 90 days of storage. The E1 - LO emulsion (with tobacco seed oil and 0.5% lemongrass essential oil) had a neutral pH value after 48 days, the same as the E2 emulsion (with grape seed oil). The E2 - LO emulsion (with grape seed oil and 0.5% lemongrass essential oil) maintained its alkaline character for 48 days and became neutral, just as the other emulsions. Grape Seed Oil (*Vitis Vinifera*, L) Facial Cream was reported by Supriadi and Khoirin, (2022) to have a pH of 7 on the day 0 and 6 after the 7th day of storage, which is comparable to the results for the tobacco seed oil emulsion.

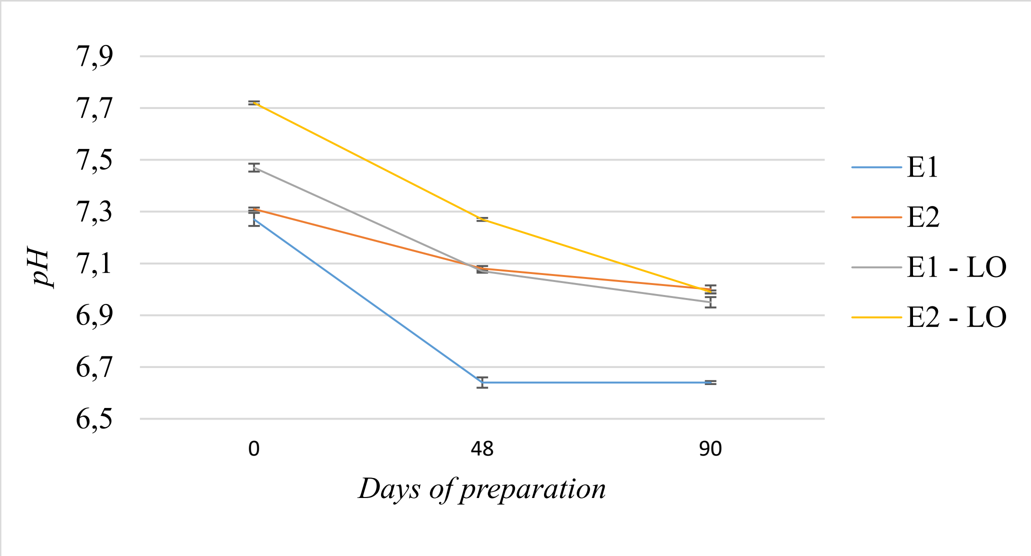


FIGURE 3. Change in pH values in the emulsions over the period of storage
E1 – tobacco oil emulsion; E1-LO – tobacco oil emulsion with 0.5% lemongrass oil;
E2 – grape oil emulsion; E2-LO – grape oil emulsion with 0.5% lemongrass oil
*Mean ± SD, n=3

The refractive index of the emulsions was also analyzed. The index of all emulsions exceeded 1.600, which was higher than that of the oils. A cosmetic emulsion with a refractive index over 1.6 typically indicates that the product is capable of manipulating how light interacts with the skin. This high refractive index suggests that the emulsion contains ingredients with significant light-bending abilities. Such emulsions could be used to reduce the appearance of fine lines, wrinkles, and other imperfections or reduce shine on oily skin and improve coverage (Orchard *et al.,*2019).

The oxidative stability of the examined emulsions was over 2.00 h as E1 - LO (emulsion with tobacco seed oil and 0.5% lemongrass essential oil) exhibiting the highest oxidative stability of 3.85±0.02 h. The stability of E2 – LO (emulsion with grape seed oil and 0.5% lemongrass essential oil) was lower than that of E2 (emulsion with grape seed oil). The emulsions demonstrated comparable oxidative stability, indicating that lemongrass essential oil could be used as a natural preservative for tobacco-based emulsions (Lukić *et al.,* 2021). The results presented differing effects of lemongrass essential oil on the grape seed oil and tobacco seed oil-based emulsions. While lemongrass oil increased the oxidative stability of the tobacco-based emulsion, its effect on grape seed oil- based emulsion was minimal or slightly reduced (Table 4).

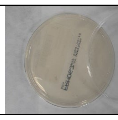
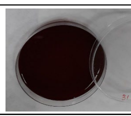
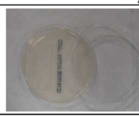
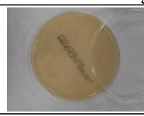
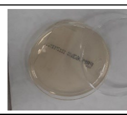
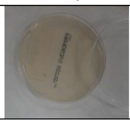
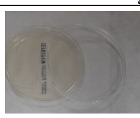
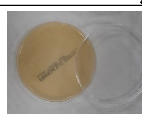
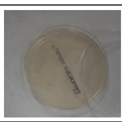
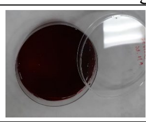
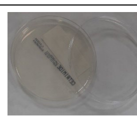
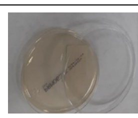
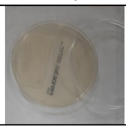
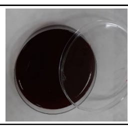
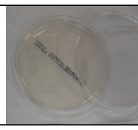
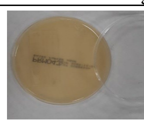
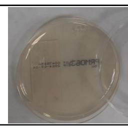
The neutral pH of the emulsions can be attributed to their higher water content and the absence of a pH regulator. For skin application, a pH between 5.5 and 6.5 is considered the optimal; however, many products, especially those designed for cleansing and moisturizing, have a neutral pH (Farris, 2018).

The high moisture content in the emulsions is primarily due to water being the continuous phase. However, water-based formulations are prone to the growth of mold and yeast (Shkreli *et al*., 2022). The emulsions were tested against Gram-negative and Gram-positive bacteria. The total number of aerobic mesophilic microorganisms was less than 10 cfu/g for three of the emulsions, as shown in Table 5. Only the E2 emulsion (with grape seed oil) had a higher count of aerobic mesophilic microorganisms $1.1 \times 10^4 \pm 6.4 \times 10^3$ cfu/g, which could be caused by contamination during preparation, storage conditions or packaging. Molds and yeasts were below 10 cfu/g in all emulsions. *Escherichia coli, Pseudomonas aeruginosa, Staphylococcus aureus,* and *Candida albicans* were not detected in any of the examined emulsions. The addition of lemongrass essential oil helped suppress the development of microbial colonies in the E2 - LO emulsion (Lukic *et al*., 2021).

Based on the results of the study, the tobacco seed oil emulsions could be considered suitable for use as moisturizers. Most commercially-available moisturizers contain around 80% water and are oil-in-water

(O/W) emulsions. These products can help reduce the appearance of fine lines and wrinkles while giving the skin a dewy, hydrated appearance (Farris, 2018). A similar application has been provided for grape seed oil-based emulsions (Selwyn and Govindaraj, 2023).

TABLE 5. Detection of microorganisms in emulsions with tobacco and grape seed oils

Emulsion	Total number of aerobic mesophilic microorganisms, cfu/g	Molds and yeasts, cfu/g	*Escherichia coli*	*Pseudomonas aeruginosa*	*Staphylococcus aureus*	*Candida albicans*
E2	$1.1\times10^4 \pm 6.4\times10^3$	<10	not detected in 1g	not detected in 1g	not detected in 1g	not detected in 1g
						
E2-LO	<10	<10	not detected in 1g	not detected in 1g	not detected in 1g	not detected in 1g
						
E1	<10	<10	not detected in 1g	not detected in 1g	not detected in 1g	not detected in 1g
						
E1-LO	<10	<10	not detected in 1g	not detected in 1g	not detected in 1g	not detected in 1g
						

*Mean ± SD, n=3; E1 – emulsion with tobacco seed oil; E1 – LO emulsion with tobacco seed oil and 0.5% lemongrass essential oil; E2 – emulsion with grape seed oil; E2-LO emulsion with grape seed oil and 0.5% lemongrass essential oil

4. CONCLUSIONS

The study demonstrates that tobacco seed oil holds significant potential as a carrier oil in cosmetic formulations, comparable to the well-established grape seed oil. Both oils exhibit favorable physical and chemical properties, such as low peroxide values, acceptable acid values, and high oxidative stability, making them suitable for cosmetic applications. Tobacco seed oil, in particular, showed a lower peroxide value (2.15 ± 0.37 meqO_2/kg) compared to grape seed oil, indicating better stability and suitability for use in cosmetic products. Although tobacco seed oil had a higher acid value (3.26 ± 0.04 mg KOH/g) due to its unrefined nature, it remained within the recommended limits for cosmetic oils (< 4.0 mg KOH/g).

The fatty acid composition of both oils, dominated by linoleic acid (C18:2), aligns with the lipid profile of human skin, making them effective in maintaining the skin's barrier function and hydration. Tobacco seed oil, with a fatty acid ratio of 1:1:4 (SFA:MUFA:PUFA), closely resembles the ideal lipid distribution for skin health, even more so than grape seed oil (1:2:4). Additionally, the high tocopherol content in both oils, particularly γ-tocopherol in tobacco seed oil and α-tocopherol in grape seed oil, provides natural antioxidant properties, protecting the oils from oxidative degradation and enhancing their stability in cosmetic formulations.

The emulsion creams formulated with tobacco seed oil exhibited excellent physical properties, including high moisture content (> 80%), neutral pH, and a refractive index above 1.600, which is beneficial for reducing the appearance of skin imperfections. The oxidative stability of tobacco-based emulsions was further enhanced by the addition of lemongrass essential oil, which also acted as a natural preservative, inhibiting microbial growth. In contrast, grape seed oil-based emulsions showed slightly lower oxidative stability and higher microbial counts, suggesting that tobacco seed oil may offer superior performance in certain applications.

Overall, the findings suggest that tobacco seed oil is a viable and effective alternative to grape seed oil in cosmetic formulations, particularly for moisturizing and anti-aging products. Its favorable properties, combined with its potential for sustainable utilization of tobacco by-products, make it a promising ingredient for the cosmetic industry. Further research could explore its long-term stability, sensory properties, and efficacy in various cosmetic applications to fully realize its potential.

RESEARCH DATA POLICY

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DECLARATION OF COMPETING INTEREST

The authors of this article declare that they have no financial, professional or personal conflicts of interest that could have inappropriately influenced this work.

AUTHORSHIP CONTRIBUTION STATEMENT

L. Stoyanova: Conceptualization, Formal analysis, Funding acquisition, Investigation, Methodology, Project administration, Writing – original draft.

M. Angelova-Romova: Conceptualization, Formal analysis, Funding acquisition, Investigation, Methodology, Project administration, Writing – review & editing.

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