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ORIGINAL: Development of a Formulation of Vanishing Cream Containing Wheat Germ Oil with Safe Preservation, and Measurement of Antioxidants in the Cream by a Rapid Method

Seyed Morteza Robatjazi School of Chemistry and Chemical Engineering, Malek Ashtar University of Technology, Tehran, Iran.
Narges Goodarzi School of Chemistry and Chemical Engineering, Malek Ashtar University of Technology, Tehran, Iran.
Hossein Ali Ettehadi School of Chemistry and Chemical Engineering, Malek Ashtar University of Technology, Tehran, Iran.
Samira Samadieh Institute of Biochemistry and Biophysics, University of Tehran, Tehran, Iran.
Mehdi Zeinoddini School of Chemistry and Chemical Engineering, Malek Ashtar University of Technology, Tehran, Iran.

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Correspondence:
Seyed Morteza Robatjazi, School of Chemistry and Chemical Engineering, Malek Ashtar University of Technology, Tehran, Iran.
Email: s_m_robatjazi@mut.ac.ir
ORCID: 0000-0001-5334-6134

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ABSTRACT

Introduction: Wheat germ is a by-product of the wheat mill and contains 10 to 15% of the oil. The use of wheat germ oil in cosmetics is because this oil contains ceramide that prevents skin aging and can preserve the skin.

Material and Methods: A vanishing oil in water (O/W) cream based on wheat germ oil was made and formulated. The antioxidants of the cream were extracted, and the best extraction conditions were obtained by the Taguchi method. Four factors of temperature, time, 2,2-diphenyl-1-picrylhydrazyl (DPPH), and round per minute (RPM) were investigated at three levels. To maintain consumer health, carcinogenic preservatives such as parabens were removed from the formulation, and a new composition of methylpropanediol, capryl glycol, and phenyl-propanoldiol (OMP) was used in the formulation. At microbial testing, two standard strains of Staphylococcus aureus and Pseudomonas aeruginosa were selected as representative of gram-positive and gram-negative bacteria. The stability of the antioxidants of wheat germ oil in the cream structure was studied.

Results: In the extraction of the antioxidants from the cream structure, the highest impact was related to the DPPH factor (78%) and temperature (14%), respectively. A growth inhibition zone of 24mm diameter was observed for S. aureus and 16mm for P. aeruginosa. The wheat germ oil cream was completely stable at 37°C after 30 days.

Conclusion: A vanishing herbal cream was formulated base on OMP composition with a very good antimicrobial activity. A rapid extraction and measurement method of the cream antioxidants was established and optimized based on DPPH.

Introduction

Wheat germ oil consists of non-polar lipids, glycolipids, phospholipids, alcohols, esters, aldehydes, tocopherols, alkanes, sterols, 4-methyl sterols, hydrocarbons, pigments, and volatile components. Using wheat germ oil in cosmetics is due to the presence of ceramides that prevents or slows down the signs of skin aging. The moisturizing properties of ceramides can be increased by using wheat germ oil with a high content of vitamin E. Decreasing the amount of essential fatty acids

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lead to Epidermal thickening the epidermis to thicken (1, 2). Neutrophil elastase, a protease serine, plays an important role in inflamematory processes and structural changes in the connective tissues that its activity can be inhibited by chain fatty acids and their derivatives (3). It is also a good antioxidant due to its tocopherol (1300-2700 mg/kg) and carotenoid (56 mg/kg) and plays an important role to prevent disease (4). Ferulic acid is another natural antioxidant found in wheat germ oil (5). Extraction of wheat germ oil tends to reduce free radicals 2, 2-diphenyl-1-1 pericyl hydrazil tendency is comparable with synthetic antioxidants and this such as butylated hydroxytoluene and butylated hydroxyanisole (6). The antimicrobial activity of wheat germ extract has been tested on pathogenic bacteria and the result shows *Escherichia coli*, *Salmonella enterica*, *Listeria monocytogenes*, and *Staphylococcus aureus* were the most sensitive species, respectively (7). Methyl propanediol, caprylyl glycol, and Phenyl propanol are surfactants that protect our product from a variety of bacteria, yeasts, and fungi in oil in water (O/W) emulsions without pH restriction (8, 9). These compounds can perform the preservative function effectively and safely (9). In cosmetic Ingredients, methyl propanediol and phenylpropanediol are commonly used as solvents; caprylyl glycol acts as emollient, humectant, and hair conditioning; however, all of them show an important antibacterial activity, and due to their antibacterial properties in the cosmetic industries is widespread used them (10-12).

Methylpropanediol, caprylyl glycol, and phenylpropanediol are excellent alternatives to carcinogenic compounds such as paraben. Parabens hurt human health and affect the expression of collagen in human skin fibroblasts. Parabens also intensify the harmful effects of UVB radiation on human skin cells, leading to increased production of free radicals, nitric oxide synthesis, and lipid peroxidation. Lately, the use of parabens has decreased among pharmaceutical companies (13, 14).

The purpose of this work was to formulate a vanishing herbal O/W cream. The advantages of this kind of emulsion are lacking greasy appearance, fast skin absorption, and better organoleptic properties than water-in-oil emulsions. Herbal creams have several advantages over other creams. These creams have no side effects. In this work, new herbal vanishing O/W cream-based drug formulations were prepared for the skin moisturizer with wheat germ oil. Also, Vitamin E was extracted from the cream structure, and the best extraction conditions were obtained by the Taguchi method.

Methods

In this study, wheat germ oil was prepared from the Malek Ashtar University of Technology. Alpha-tocopherol and 2, 2-diphenyl-1-picrylhydrazyl (DPPH) were purchased from Sigma-Aldrich Company in high purity grade. Stearic acid, methyl paraben, butylated hydroxytoluene (BHT), butylated hydroxyanisole (BHA), 2-propanol, pure ethanol, OMP compound (methylpropanediol, caprylyl glycol, and phenylpropanediol) were purchased from Merck company. French rose essential oil was obtained from Dermosoft Company. Also, two standard strains of *Staphylococcus aureus* (ATCC 25923) and *Pseudomonas aeruginosa* (ATCC 15692) were used for antimicrobial tests.

Preparation of wheat germ oil

Wheat germ (Triticum aestivum type) was firstly heated to 70°C and wheat germ oil was extracted by cold compress method. The extraction was carried out in a continuous system. The cold pressing was performed in a laboratory prototype apparatus (Iran cold pressing- 85 MM Express) (15).

Formulation of the vanishing cream

The ingredients of the oil and aqueous phases separately were mixed in a beaker in the water bath 70°C. The samples were mixed with a mechanical stirrer (Heidolph) to form a uniform suspension. The oil phase was gradually added to the aqueous phase and

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mixed for 30 minutes at a speed of 600 rpm by the mechanical stirrer. Then, wheat germ oil, the essences, and vitamins were added as antioxidants at 45°C and homogenized with a Homogenizer (Heidolph) for 10 minutes. The initial formulation of the vanishing O/W cream was shown in ***Table 1***.

Table 1. Initial formulation of the vanishing O/W creams

Materials	Percentage (W %)				
	a	b	c	d	e
Stearic acid	5	5	5	5	5
Cetyl alcohol	1	1	1	1	1
Glycerol mono stearate	1	1	1	1	1
Isopropyl Mir Stearate	1	1	1	1	1
French rose essential oil	0	0.25	0.3	0.2	0.15
Glycerol	5	5	5	5	5
Triethanolamine	0.50	0.5	0.4	0.35	0.5
OMP[1]	0	2.5	3	3.5	4
BHA[2]	0.02	0	0	0	0
BHT[3]	0.1	0	0	0	0
Methyl paraben	0.2	0	0	0	0
Propyl paraben	0.02	0	0	0	0
Vitamin E	0	0.02	0.03	0.04	0.05
Wheat germ oil	0	4	5	7	3
Distilled water	to 100	to 100	to 100	to 100	to 100

1: Methylpropanediol, capryl glycol, phenylpropanol diol, 2: Butylated hydroxyanisole, 3: Butylated hydroxytoluene

Measurement of vitamin E

Vitamin E was measured by the orthoquinone method. 0.2 mg of vitamin E was dissolved in 2 ml of absolute ethanol. Then, 0.2 ml of concentrated nitric acid was added to the solution. The solution was heated in a water bath for 5 minutes and in the presence of vitamin E, the color of the solution changed from yellow to brick red due to the formation of orthoquinone.

In addition, vitamin E was measured by the HPLC. The fat-soluble vitamins of the samples can be separated and identified in reverse columns using a mixture of water and methanol as the mobile phase (16). 0.2 mg of vitamin E was dissolved in 2 ml of absolute ethanol. Then, 0.2 ml of concentrated nitric acid was added to the solution. The standard curve of vitamin E (alpha-tocopherol) is shown in ***Figure 1***.

The anti-radical activity of oil in 2-propanol was investigated using DPPH (2, 2-diphenyl-1-picrylhydrazyl). In this method, alpha-tocopherol was used as a control. 1485 µL of 100 µmol/L DPPH was added to 15 mg of oil in a microtube. After 10 min incubation in the dark at 25°C, the absorbance was determined at 517 nm. A standard curve was determined by different alpha-tocopherol concentrations. The amount of alpha-tocopherol obtained was approximately equivalent to that determined by HPLC (16, 17).

Protein determination in wheat germ oil

The amount of protein in wheat oil was measured using the Kjeldahl nitrogen method (18).

Emulsion type

The staining was performed to identify the emulsion being the O/W. After staining using Coomassie Brilliant Blue, a slide of it was examined under a microscope with a magnification of 40% using a slide. The dye used because it is soluble in water is in the blue phase of the emulsion, so if colorless micro-globules are seen in a blue background, the emulsion is O/W, and if the blue micro-globules are in a colorless background, the emulsion is a water-in-oil.

pH control

The pH of the cream was measured by diluting them with distilled water. (Ratio 9: 1). The laboratory benchtop pH meter (model PTR 79 from ZagChmie Company) was used.

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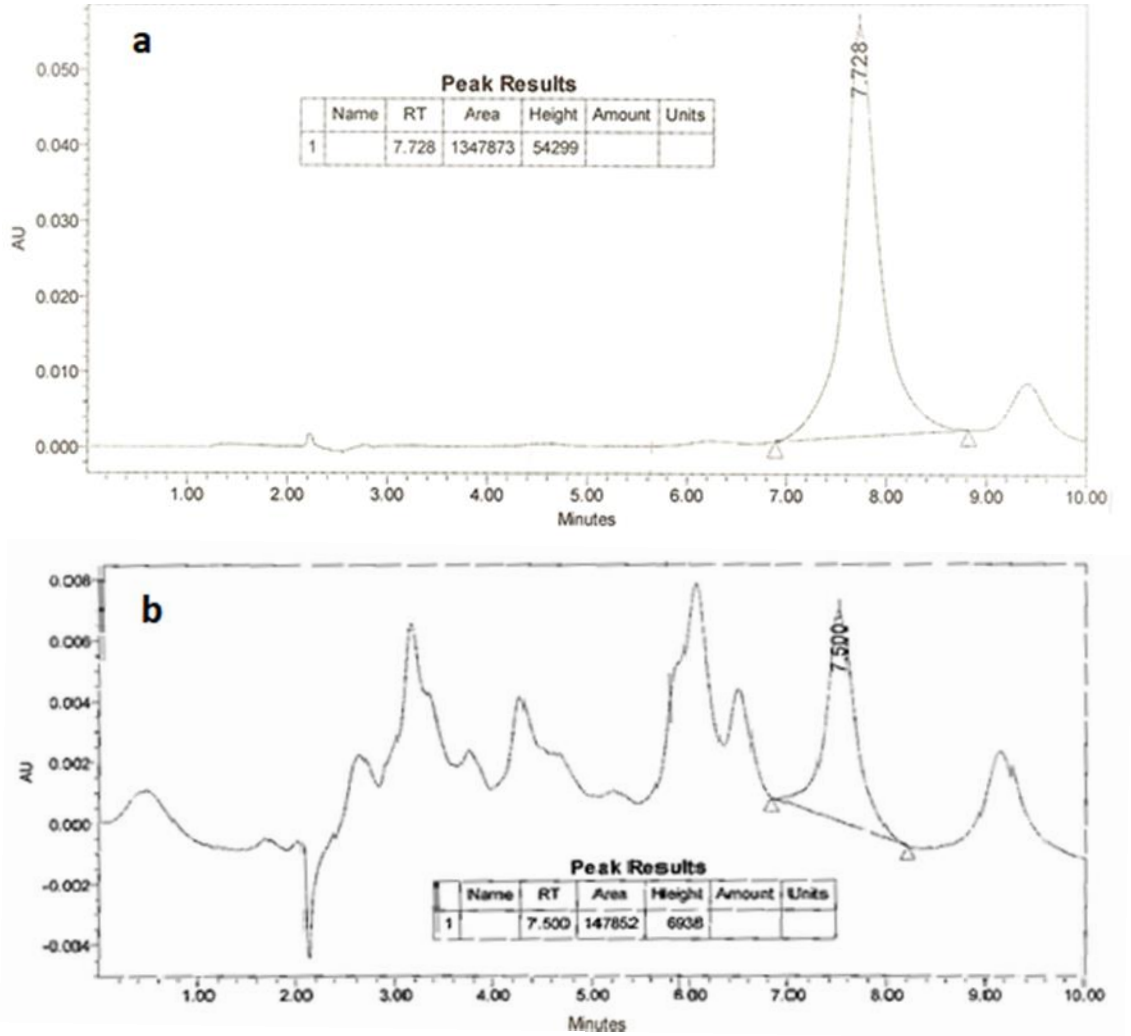

Figure 1. Graphs of the vitamin E concentration using HPLC. a: standard curve of alpha-tocopherol, b: wheat germ oil.

Particle size of the cream suspension

The particle size of the cream suspension was determined by dissolving a small amount of the cream in distilled water and then observed under a microscope. Finally, to determine the structure of the cream, the average particle size was determined by a particle size analyzer (PSA). Properties of microemulsion flow of cream (100% w) were measured by modular compact rheometer (MCR 300 Anton Paar model) at 25°C to measure shear stress and viscosity as a function of shear velocity and determine the flow behavior of samples in a time interval of 10 minutes, the shear velocity of 2 reached 100 s-1. Then, to describe the flow behavior, the curve of these results was plotted.

Optimization of extraction conditions for antioxidants using the Taguchi method

For optimization extraction conditions, the experimental design was performed by the Taguchi method using Qulitek-4 software. The optimal conditions were obtained from investigation of four factors at three levels (L9) that were shown in ***Table 2***. In this experiment, factors of temperature, time, DPPH, and RPM were examined in three-level. The experiments were repeated three times. The contribution of the influence of each factor was obtained. The parameters investigated from the ANOVA analysis and the contributions of each factor in the experimental design were determined.

The extraction method of antioxidants of wheat germ oil from vanishing O/W cream

The cream and solvent of 2-propanol were

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mixed one by one and vortexed for 1 minute and then placed in a sonicator bath for 15 minutes. The sonicated mixture was centrifuged at 6000 g for 10 minutes. The clear solution was removed and poured into another microtube. In another microtube, mix 500 µL of the extracted solution with µL500 of DPPH stock and incubate in the dark for 30 minutes. The incubated solution was then transferred to a cuvette and by observing the change in color of the solution from purple to yellow, the absorbance of the sample was determinate by the spectrophotometer at 517 nm. As a control, cream with the same formulation but without germ oil and with alpha-tocopherol was used.

Table 1. The factors and their levels used in the Taguchi experimental design for optimization of antioxidants extraction conditions

Parameter	Level 1	Level 2	Level 3
Temperature (°C)	20	25	30
Time (min)	10	30	60
DPPH (µL)	500	600	700
RPM[1] (r/min)	100	150	200

1: Round per minute

Evaluation of Antimicrobial Activity

Two standard strains of *Staphylococcus aureus* and *Pseudomonas aeruginosa* were selected and the antimicrobial activity of the new combination of methyl propanediol, caprylyl glycol, and phenyl propanediol was investigated (6). A new combination was performed using the diffusion method (19).

Results

The cream was perpetrated based on the different formulations. The appearance of the cream was investigated for the quality, efficacy, consumer appeal, and shelf-life of formulations. First, it was yellow and after homogenization, it was milky in color. It was without stickiness and roughness.

The pH of the cream with different formulations was measured. The results related to pH measurement in different formulations were shown in ***Table 3***. Using dilution and solubility test in Coomassie Blue dye, the cream structure was obtained oil-in-water. No two-phase was observed in the mechanical stability test. Natural antioxidants such as alpha-tocopherol can be replaced with synthetic antioxidants such as butyl-hydroxy-toluene and butyl-hydroxy-anisole.

Statistical analysis has shown that the highest particle size is 0.5-1 µm and there are no particles larger than 3 µm in the cream (***Figure 2***).

Observation of growth aura in the bilateral culture medium of *Staphylococcus aureus* and *Pseudomonas aeruginosa* was shown in ***Figure 3***. The inhibition zone diameter was 24 mm for *Staphylococcus aureus* and 16 mm for *Pseudomonas aeruginosa*.

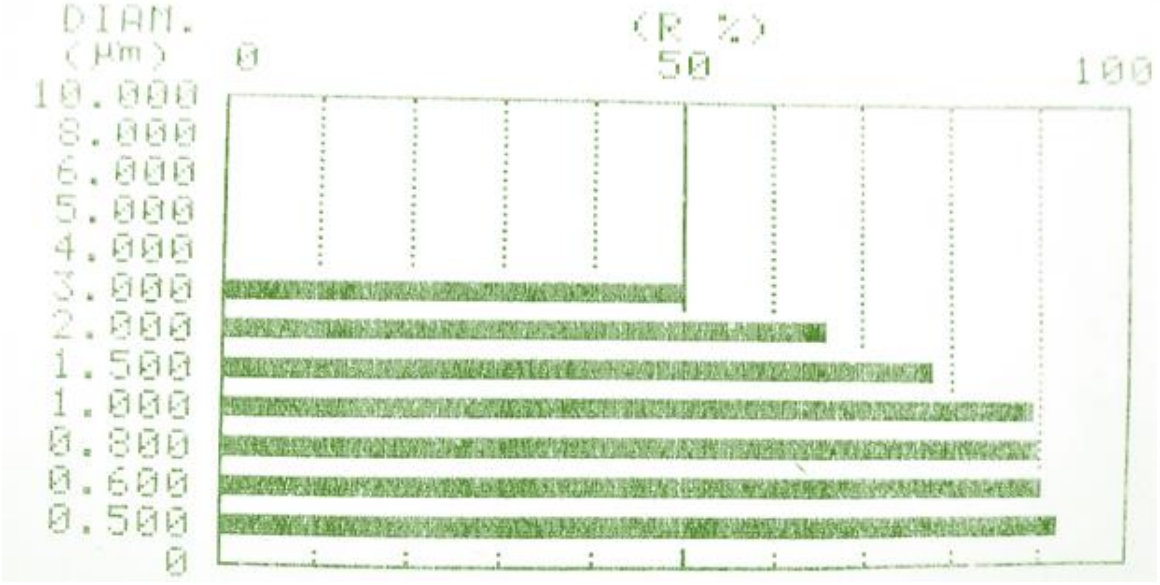

Figure 2. Bar chart of particle size in the optimal cream formulation.

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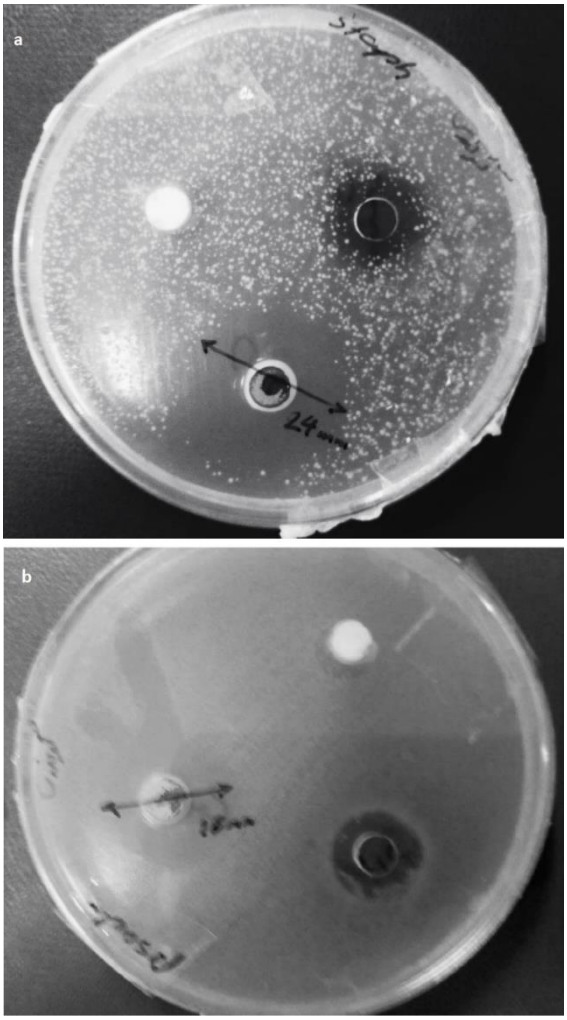

Figure 3. Observation of growth aura in bilateral culture medium. a: *Staphylococcus aureus*, b: *Pseudomonas aeruginosa*.

The results of the stability of vanishing O/W cream containing vitamin E, at 37°C, show that with increasing temperature, the stability of vitamin E decreased and vitamin E in vanishing O/W cream at 4°C is more stable than 37°C. The cream was stored for 3 months at 8°C and 37°C. The cream was completely stable without discoloration and after this time, the cream did not have a pungent odor, which proved that wheat germ oil was not oxidized.

The antioxidant was extracted from the structure cream by a new and inexpensive spectrophotometric method using alpha-tocopherol as a control. To achieve the best extraction conditions, the experimental design was performed by the Taguchi method, 4 factors of temperature, time, DPPH and, rpm were investigated in 3 levels. The results obtained from the ANOVA analysis showed that the highest contribution was related to factors with DPPH 78% and temperature with 14% in the Taguchi experimental design (***Table 4***).

The stability of alpha-tocopherol and the antioxidants in O/W cream was investigated at 4 and 37°C. The results are shown in ***Figure 4***.

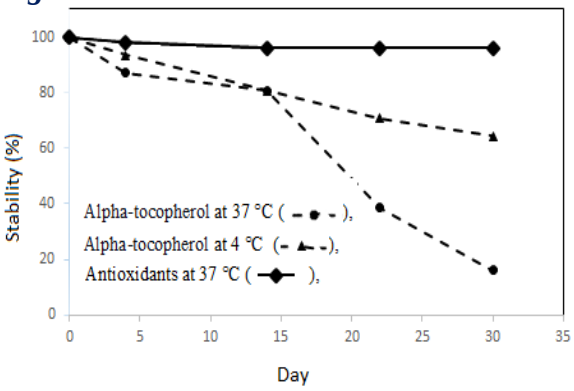

Figure 4. Stability of antioxidants of the vanishing cream of wheat germ oil.

Table 4. The ANOVA analysis and the contribution of each factor in the Taguchi experimental design

Parameter	DOF	Sum of Sqrs.	Variance	Pure Sum	Percent
Temperature	2	23.682	11.841	23.682	14.031
Time	2	6.518	3.259	6.518	3.862
DPPH	2	132.791	66.395	132.791	78.675
RPM	2	5.79	2.895	5.79	3.43
Total	8	168.783	-	-	100%

Discussion

In this study, cream with wheat germ oil was studied. Alpha-tocopherol was used in the cream formulation as a control sample. Due to its anti-inflammatory and therapeutic properties and high levels of essential fatty acids and high protein in wheat germ oil, which is rich in vitamin E, this valuable effective substance was selected for use in the

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formulation. Because the active ingredient is wheat germ oil, the ingredients used at the base of the formulations were all hydrophobic and caused an unpleasant feeling for the consumers due to the increase of the fat phase. Also, in the past, chemical presservatives as parabens have been used that have many side effects. According to today's needs and the research that has been done on the ingredients of the cream and its effects over time, it is imperative to review used formulations. Therefore, in this study, it was decided to choose a formulation that has a lower fat phase and is absorbed faster, and eliminates the unpleasant feeling of fat on the skin. As a result, the vanishing O/W cream was selected based on wheat germ oil. To protect the health of consumers, carcinogenic preservatives such as parabens were removed from the formulation and a new compound (OMP) was used in the formulation. Vitamin E replaced chemical antioxidants and the final formulation of O/W cream was obtained with pH 7. The final formulation of the cream is shown in ***Table 5***.

Table 5. Optimal O/W cream formulation with wheat germ oil

Materials	Percentage (W %)
Stearic acid	5
Cetyl alcohol	1
Glycerol mono stearate	1
Isopropyl Mir Stearate	1
French rose essential oil	0.3
Glycerol	5
Triethanolamine	0.5
OMP[1]	3
Vitamin E	0.03
Wheat germ oil	5
Distilled water	To 100

1: Methylpropanediol, capryl glycol, phenylpropanol diol

Herbal creams do not have any of the side effects of chemical creams. Because parabens have been found in breast cancer cells in the cosmetics industry, the substitution of this compound is necessary for health. OMP compound without pH limitation protects our product against a variety of microorganisms, including gram-positive and gram-negative bacteria, yeast, and fungi, and is an excellent alternative to carcinogenic compounds such as parabens. For measurement of stable rheological properties of emulsion, the viscosity of 100 points (2 - 200 s) at Constant temperature (25 ^{o}C) was obtained using a rheometer that was shown in ***Figure 5***. The range of changes of viscosity is 29600-42400 (cp) which firstly shows the non-Newtonian fluidity (which is the main feature of the cream) and secondly the high (μ) with a phase with a lot of aqueous phases that retain high moisture.

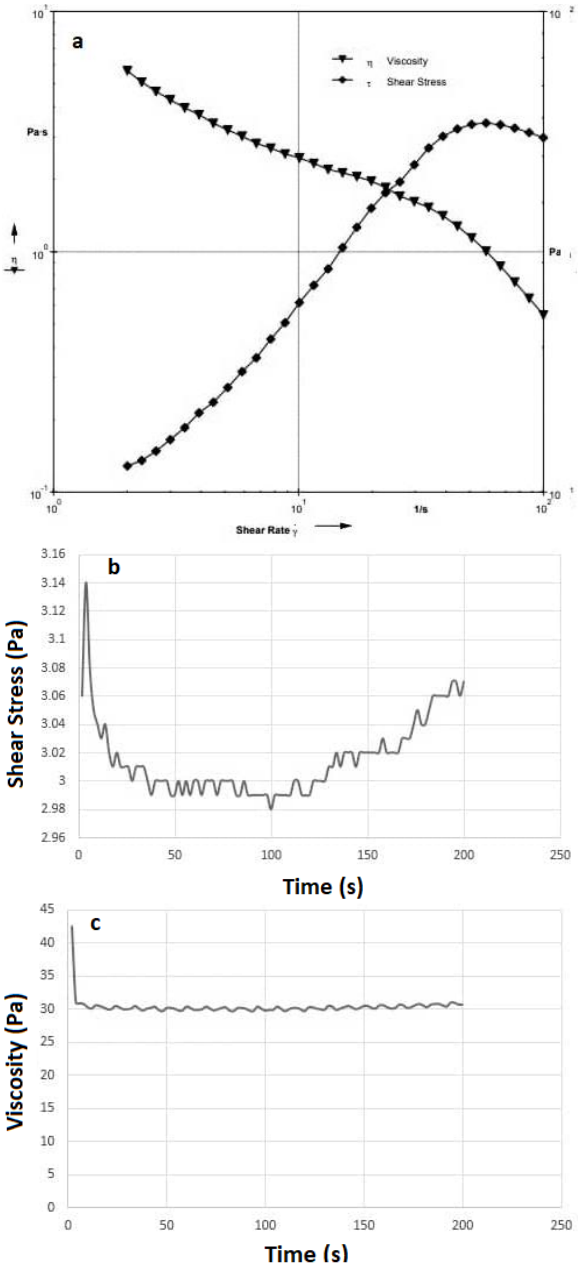

Figure 5. Properties of micro emulsion flow of vanishing O/W cream based on wheat germ oil at 25°C. a: diagram of shear stress changes from 1 to 200 seconds, b: diagram of viscosity changes from 1 to 200 seconds, c: graph of viscosity changes over shear stress from 1 to 200 seconds.

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For extraction of antioxidants by laboratory methods, the 2-propanol solvent was selected because, in the PSA test, the 2-propanol solvent was used as a blank. After dispersing the cream sample in a 2-propane solution, the particle size was measured from 1 to 13.15 µm. This indicates that 2-propanol can bind fat particles and form large micelles containing vitamin E and antioxidants. Increasing vitamin E solubility in non-ionic surfactants and protection of vitamin E from Oxidation has been reported (20). A hydrophobic compound may prefer droplets of O/W emulsion to the high-volume oil phase. In such cases, micro-emulsions can be used to extract solutes from a solution. This compound, with wheat germ oil, which has antimicrobial properties, can be used as a safe compound in the formulation.

The results showed, a high amount of 6178.35 mg/l of protein in wheat germ oil that indicates, which is very useful and valuable for the skin. A 7.5 IU/g of vitamin E, which acts as an antioxidant, was measured by HPLC. The results showed that the best case for extraction of the antioxidant of the cream was 10 minutes at a temperature of 30°C and a volume of 500 µl of DPPH and rpm of 150 r/min (***Table 6***). Because DPPH is an analytical material and is unstable in environmental conditions, it is recommended to prepare a daily test for each time to reduce individual and system errors.

Table 6. Optimal conditions obtained from the ANOVA analysis

Parameter	Parameter value	Level
Temperature (°C)	30	3
Time (min)	10	1
DPPH (µL)	500	1
RPM[1] (r/min)	150	2

The results of stability of O/W cream showed that alpha-tocopherol decreased with increasing temperature. Alpha-tocopherol is more stable in O/W cream at 4°C than at 37°C.

The results showed that the increase in temperature to 37°C did not affect in antioxidants of the wheat germ oil vanishing cream but the amount of alpha-tocopherol has drastically decreased in the cream (***Figure 4***). The antioxidants of the cream were completely stable and no decrease in activity was observed at 37°C. According to the results, the highest stability of antioxidants was obtained for vanishing cream containing wheat germ oil.

Conclusion

The most common method of measuring antioxidants is HPLC. This method is one of the most expensive methods of analysis and requires a specialist. While spectrophotometry for measuring antioxidants is a rapid and cheap method and does not require special equipment, so the set-up of this method in pharmaceutical laboratories is recommended. The antioxidants were extracted from the cream structure and measurement using a new and inexpensive spectrophotometric method using alpha-tocopherol as a control. To achieve the best extraction conditions, the experimental design was performed by the Taguchi method.

In this study, a vanishing O/W cream based on wheat germ oil was formulated. Due to its anti-inflammatory and therapeutic properties and high levels of essential fatty acids and high protein in wheat germ oil, which is rich in vitamin E, this valuable effective substance was selected for use in the formulation. Vitamin E replaced the chemical antioxidants and the final formulation of the pH 7 cream was obtained. OMP composition has shown very good antimicrobial activity and it has been a good candidate to replace the paraben-preservative compounds in cream formulations. The average particle size of the emulsion was less than 3 micro and close to 500 nanometers, which indicates the stability of the cream in the thermodynamic state and the good ability of the cream particles to spread and accelerate the penetration of the cream on the skin.

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Conflicts of interest

The authors declare no conflict of interest.

Authors' contributions

All authors have intellectually committed to the study design and process. The final manuscript was revised and accepted by all authors.

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