Optimization of rice bran oil nanoemulgel as anti-aging using Box–Behnken design

Tantri L. Nareswari, Farah D.Z. Salsabillah, Syaikhul Aziz

Department of Pharmacy, Faculty of Science, Institut Teknologi Sumatera, South Lampung, Indonesia

Correspondence to Tantri L. Nareswari, MSFarm., Department of Pharmacy, Faculty of Science, Institut Teknologi Sumatera, South Lampung 35365, Indonesia. Tel: +62 721 803 0188, +62 721 803 0189; fax: (0721) 8030189; e-mail: tantri.nareswari@fa.itera.ac.id

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Background
The accelerated aging caused by reactive oxygen species is a major concern to many people. Antioxidants present in natural products like rice bran oil (RBO) absorb and neutralize free radicals, improving skin appearance and slowing the aging process. However, RBO has limited use on the skin due to greasy sensation, oxidation, and low penetration.

Objective
This study aimed to develop a RBO nanoemulgel and evaluate its anti-aging effects on volunteer skin.

Patients and methods
Nanoemulsion optimized using Box–Behnken design to optimize RBO (x_1), surfactant (x_2), and cosurfactant (x_3) factors towards transmittance response. Nanoemulsion prepared using high-pressure homogenizer and ultrasonication methods.

Results and conclusion
The optimal formula contained 2% oil, 14.732% tween 80, and 1.227% span 80, with characterization of 96.6% transmittance, particle size of 17.8±0.1 nm, polydispersity index of 0.51±0.00, zeta potential of −3.56±0.70, and pH of 6.19 ±0.05. The optimal formula was further incorporated with CMC-Na base and evaluated it anti-aging effects on upper arm skin volunteer. After 4 weeks of RBO nanoemulgel usage, there was a significant increase on oil content and elasticity parameters on skin volunteers, but not on moisture content. This study highlights the potential of the optimized nanoemulgel formula as an anti-aging solution, addressing signs of aging.

Keywords:
Box–Behnken design, nanoemulgel, nanoemulsion, rice bran oil, skin-aging

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Introduction

Premature aging has become a major concern for many people because it effects the appearance. The process of aging is defined by the development of wrinkles, pigmented, and dry skin, as well as a progressive decline in the function and capacity of the skin as a result of internal and external influences [1]. Genetic factors influence intrinsic structural changes that come naturally with aging, while extrinsic variables like ultraviolet (UV) radiation from sun exposure can also play an important role. When skin is exposed to sunlight, reactive oxygen species (ROS) which are produced may disrupts the antioxidant balance, causing oxidative stress, tissue damage, or skin aging prematurely [1–3].

Antioxidant are compounds that can absorb and neutralize free radicals, by donating a free electron or accepting an unstable electron, hence inhibits oxidative processes [3]. Antioxidants can offer additional protection against UV radiation exposure, which help to delay the aging process and enhance the appearance of the skin [3,4]. Natural antioxidants from various plants have been proven effective in preventing oxidative damage caused by free radical reactions. One of the natural product wastes that has antioxidant compounds is rice bran [5].

Rice bran is one of the by-products that is produced when the inner layer of the bran is removed from the rice during milling. Rice bran has only lately been used as animal feed, despite the fact that it contains high levels of nutrients and abundance of beneficial antioxidant substances for our bodies, including orizanol, tocopherols, tocotrienols, phystosterols, polyphenols, and squalance [6,7]. However, RBO exhibits hydrophobic properties, which makes it poorly soluble and challenging to absorb [8]. In addition, oil is easily oxidized, sticky, and uncomfortable when used, thus a novel drug delivery technique is needed to maximize its use.

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A nanoemulsion is an emulsion with a system of oil and water dispersions stabilized by surfactants and a droplet size of less than 100 nm. Nanoemulsion is distinguished by a translucent look and a high transmittance value [9]. The penetration of the active component can be increased by nanoemulsion since there is a greater potential for active substance to enter a formulation as droplet size increases [9]. Topical nanoemulsions based on antioxidants have been demonstrated to help moisturize skin because the active ingredients are evenly distributed in tiny droplets across the stratum corneum [10]. Therefore, nanoemulsion can be a platform for more efficient RBO delivery. It also inhibits oxidation, reduces stickiness, and is less greasy, all of which will improve patient acceptance.

Nanoemulsion stability is influenced by the type and concentration of cosurfactants and surfactants used [11]. To achieve the optimum concentration, optimization is required. The Box–Behnken Design method can be used for optimization since it is more efficient than other response surface methodologies [12].

This work aims to optimize the concentration of RBO, cosurfactants, and surfactants in the formation of stable nanoemulsions using the Box–Behnken design approach. The optimum formula is then incorporated to a gel basis to increase the comfort of its application to the skin. The improved flexibility, moisture content, and oil content of the nanoemulsion gel were then evaluated on the skin of human test patients.

Patients and methods

RBO was purchased from Happy Green, Indonesia; Merck, USA; Sigma-Aldrich, USA. Tween 80 (Merck), span 80 (Merck), ethanol, CMC-Na, were pharmaceutical grade. y-oryzanol were obtained from Sigma Aldrich. All other reagents and chemicals were of analytical grade.

Identification of y-oryzanol

Identification of RBO was carried out using the thin layer chromatography (TLC) method, using silica gel F_{254} as the stationary phase and n-hexane–ethyl acetate as the mobile phase (8 : 2) [13]. The sample was spotted on a TLC plate, added to a chromatographic chamber that had already been saturated with mobile phase, and allowed to elute until a predetermined limit was achieved. Compound detection was performed using UV 254 and UV 366 lamp, as well as the production of visible spots by spraying a 10% H_2SO_4 solution.

Formulation of rice bran oil nanoemulsion

The preparation of RBO nanoemulsion uses a previous studies with some modifications [14]. Briefly, RBO, tween 80, and span 80 were mixed in an Ultraturrax homogenizer for 4 min at 8000 rpm at 50°C to produce a core emulsion. The homogeneous mixture was next subjected to 8 min of ultrasonic sonication at 50°C.

Optimization of rice bran oil nanoemulsion

Nanoemulsions were optimized using the Box–Behnken design method in Minitab, version 21. The three factors used in this study were RBO concentration (x_1), tween 80 (x_2), and span 80 (x_3), and the response was the transmittance (%) of the nanoemulsion. The factors and factor levels are presented in Table 1 and the 15 experimental formulas that the design produces are shown in Table 2.

The transmittance of the RBO nanoemulsion was measured at a wavelength of 650 nm using a UV–visible spectrophotometer [15]. Aquadest was used as a blank. The optimum RBO nanoemulsion formula was determined by analyzing the transmittance percentage (%) with a target of 90–100%. The model was then verified by comparing experimental results with predictions.

Characterization of rice bran oil nanoemulsion

The optimum RBO nanoemulsion formulation characteristics, namely organoleptic, particle size,

Table 1 Rice bran oil nanoemulsion formulation factor level

		Level (%)		
No.	Factor	Low	High	Transmittance (%)
1	RBO	2	4	90–100
2	Tween 80	13.5	15.5	
3	Span 80	0.5	1.5	

Table 2 Formula of rice bran oil nanoemulsion

	Composition (% b/b)		
Formula	Rice bran oil	Tween 80	Span 80
1	2	13.5	1
2	4	13.5	1
3	2	15.5	1
4	4	15.5	1
5	2	14.5	0.5
6	4	14.5	0.5
7	2	14.5	1.5
8	4	14.5	1.5
9	3	13.5	0.5
10	3	15.5	0.5
11	3	13.5	1.5
12	3	15.5	1.5
13	3	14.5	1
14	3	14.5	1
15	3	14.5	1

zeta potential, and pH properties, were evaluated. Particle size and zeta potential was tested using a Particle Size Analyzer (Horiba SZ-100) [16].

Formulation of rice bran oil nanoemulgel

Nanoemulgel was prepared using the method from Miastkowska *et al.* [10]. Briefly, nanoemulsion was incorporated into the swelled CMC-Na gel base and stirred for 30 min until it completely mixed. The formed nanoemulgel was then tested on human volunteers for parameters of moisture content, oil content, and elasticity.

In-vivo skin hydration and viscoelasticity studies

The study was approved by the Ethics Committee of Malahayati University of Medical Sciences (3529/EC/KEP-UNMAL/V/2023) in accordance with the Helsinki Declaration. Written informed consent was obtained from each participant before the study.

Twelve volunteers willing to participate in testing, between the ages of 20 and 25, without a history of allergies or allergy-related illnesses, were selected for this study [17]. Volunteers are initially tested for product irritation with methods by Ardhany *et al.* [18] with some modifications. In brief, the irritation test was conducted on the skin using patch method. Reactions including redness, itching, and swelling of the skin were observed before use as well as 1 and 24 h after the patch was removed. During the test, volunteers were allowed to shower, but they were not allowed to apply any cosmetics to the sample region. The volunteers who showed no symptoms of irritation subsequently underwent an anti-aging test.

The anti-aging test was carried out on the upper arm of 12 volunteers with parameters including moisture content, elasticity, and oil content. The initial condition of the skin was tested before using the preparation. Nanoemulgel (as control) and RBO nanoemulgel (as sample) were applied evenly on the left and right upper arms, respectively. Volunteers applied nanoemulgel twice daily for four weeks – once before night and once before morning activities. Changes in moisture content, elasticity, and oil content following sample and control treatment were evaluated on a weekly basis using a skin analyzer (Meicet) and compared with Minitab 21 [17].

Statistical analysis

Optimization was processed by Box–Behnken design method using Minitab 21 software to obtain the optimum formulation. In-vivo skin tests were processed using two-way analysis of variance with SPSS, USA at a 95% confidence level.

Results and discussions

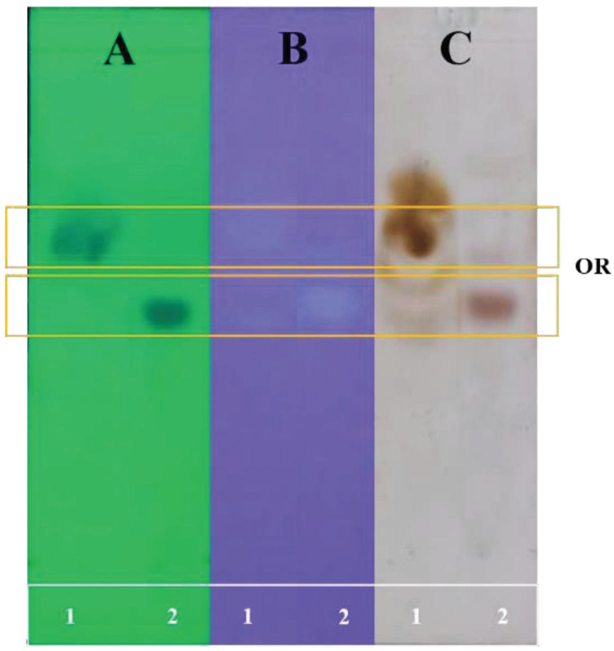
Identification of oryzanol

The TLC profile of RBO was shown in Fig. 1. Oryzanol standard produces two spots, namely the retardation factor (R_f) 0.6 and 0.4. The two spots are thought to indicate the presence of oryzanol with different spatial configurations. The same was found in a previous study [19]. This different spatial configuration can occur because of the ferulic structure of one of the oryzanol constituents. Ferulic acid molecules have both cis and trans structures, and they differ in terms of their physical properties [20]. Trans-structured molecules often have lower R_f values than cis-structured molecules [21]. This finding indicates that oryzanol was present in the RBO used in this study.

Optimization of rice bran oil nanoemulsion

Table 2 shows the results of the transmittance response of each formula. Transmittance (%) was chosen as the response because the high transmittance value, which is one of the elements influencing consumer acceptability, depicts small particle sizes [22].

Figure 1



Thin layer chromatogram of rice bran oil eluted on silica gel F254 stationary phase and n-hexane: ethyl acetate (8 : 2) mobile phase system. (a) Observation under 254 nm UV light; (b) observation under 365 nm UV light; (C) observation under visible lights with 10% H_2SO_4 spraying. 1. Rice bran oil; 2. oryzanol standard. OR, oryzanol. UV, ultraviolet.

Table 3 Analysis of variance statistical analysis on the Box–Behnken Design for rice bran oil nanoemulsion formula

Source	DF	Adj SS	Adj MS	*F* value	*P* value
Model	9	18 181.3	2020.1	4.83	0.049
Linear	3	16 532.0	5510.7	13.17	0.008
RBO	1	13 246.0	13 246.0	31.65	0.002*
Tween 80	1	212.1	212.1	0.51	0.508
Span 80	1	3073.9	3073.9	7.34	0.042*
Square	3	1432.5	477.5	1.14	0.417
RBO×RBO	1	114.4	114.4	0.27	0.623
Tween 80×Tween 80	1	738.5	738.5	1.76	0.241
Span 80×Span 80	1	753.9	753.9	1.80	0.237
2-way interaction	3	216.8	72.3	0.17	0.910
RBO×Tween 80	1	0.4	0.4	0.00	0.976
RBO×Span 80	1	198.2	198.2	0.47	0.522
Tween 80×Span 80	1	18.2	18.2	0.04	0.843
Error	5	2092.7	418.5		
Lack-of-fit	3	1681.8	560.6	2.73	0.280
Pure error	2	410.8	205.4		
Total	14	20 273.9			

RBO, rice bran oil. **P* value less than 0.05.

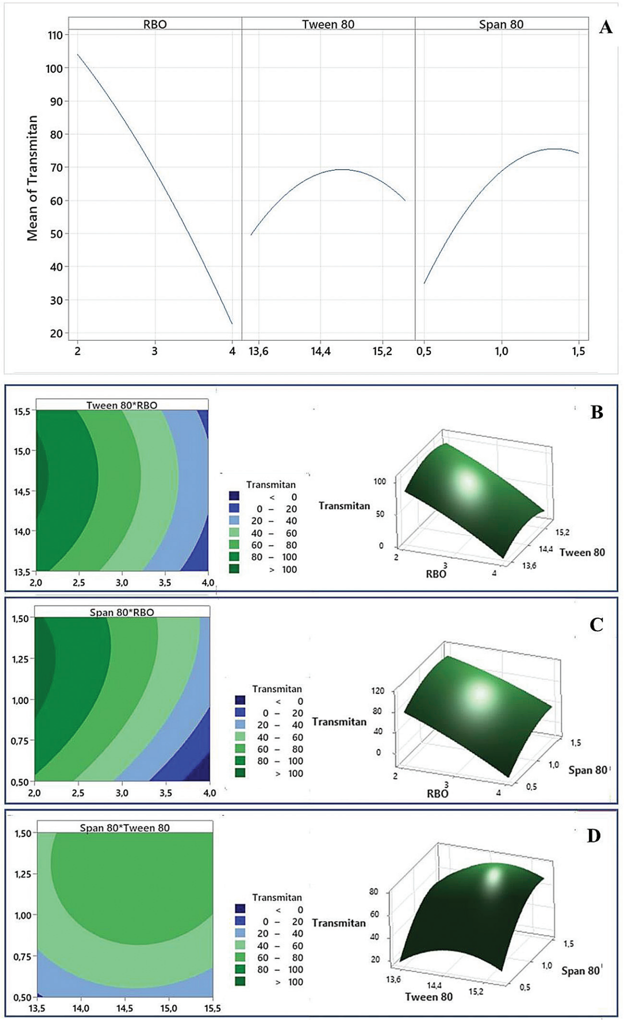
The analysis of variance analysis shown in Table 3. The r^2 value shown in the table is 89.6% indicating a good fit in the model. According to earlier study, a high value of r^2 indicated a good correlation between the experimental data and the projected value [23]. Lack of fit in the model also showed an insignificant value ($P>0.05$). In addition, model *P* value is 0.049 ($P<0.05$), which indicates a significant value. These results showed that the model was fitted.

Table 3 showed that RBO were statistically significant effect to transmittance, with a significance of 0.002 ($P<0.05$). This data was supported by the polynomial equation shown by equation (1), which showed that the RBO concentration gives an antagonistic value (minus value) to the response. This result was also in line with the factor-response description shown in Fig. 2a–c, which showed that higher concentration of RBO resulted in lower transmittance percentage.

$$\begin{gathered}\text{Transmittance}(\%) = -2913 - 17\ \text{RBO} + 412 \\ -2913 - 17\ \text{RBO} + 412\ \text{Tween 80} + 49\ \text{Span 80} \\ -5,6\ \text{RBO} * \text{RBO} - 14,1\ \text{Tween 80}* \\ \text{Tween 80} - 57,2\ \text{Span 80} * \text{Span 80} \\ -0,3\text{RBO} * \text{Tween 80} + 14,1\ \text{RBO} \\ *\text{Span 80}+4,3\ \text{Tween 80} * \text{Span 80}\end{gathered}$$

Conversely, from the equation (1), the use of tween 80 and span 80 produces a synergistic effect on the response, where increasing the concentration used will result in a high transmittance value. Span 80 as a cosurfactant also significantly affect transmittance (P=0.042, Table 3), where at a concentration of 1–1.5% it produces nanoemulsions with high transmittance (Fig. 2a and c, d). Meanwhile, the use

Figure 2



Graph of the main effects (a) and contour plots of tween 80-RBO (b); span 80-RBO (c); span 80-tween 80 (d); towards transmittance (%).

of tween 80 did not make a statistically significant contribution (Table 3), but increase on the transmittance response at a certain concentration (Fig. 2a–d). The increase in oil that lowers transmission is consistent with earlier studies [24]. The authors stated that that using high concentrations of oil can decrease the effectiveness of both surfactants and cosurfactants in lowering particle size because high oil concentrations lead to globule expansion in droplets. Higher concentration of span 80 can increase the transmittance value since it is a cosurfactant that aids surfactants in lowering the interfacial tension between the two immiscible phases [23]. In numerous trials, the cosurfactant and surfactant combination of tween 80 and span 80 has been shown to significantly reduce particle size [23,25]. However, in our study, tween 80 did not have a significant effect on increasing the transmittance value. Tween 80 can increase the transmittance at concentrations of ~14.4–15% (Fig. 2b), but at lower (13.5–14.4%) and higher concentrations (15–15.0%), the transmittance decreases. This might be caused by the used of surfactant over the optimal amount, which could lead to the development of Ostwald ripening and higher particles with more Brownian motion [26].

Verification of rice bran oil nanoemulsion optimum formula

The optimum formula is produced by processing experimental data in Minitab using the maximum transmittance response criterion. The optimal composition of a nanoemulsion is 2% RBO, 14.73% tween 80, and 1.227% span 80, with a desirability value of 1. The desirability value is a functional value that can indicate the ability of the program to predict the experiment result. A value of desirableness that is close to 1 indicates that the optimization model best matches the predictions [27].

Predictive data was verified by formulating the obtained prediction formula using the same method. The response used was transmittance (%) with maximum contains and a predictive value of 107% is obtained. The transmittance from the optimum formula that the experiment produced was 96.6 ±0.378%. This value was close to the predicted value.

Characterization of rice bran oil nanoemulsion

The organoleptic test of RBO nanoemulsion was shown in Fig. 3. The optimum RBO nanoemulsion formula resulted in a liquid that was clear, yellowish, has a low viscosity, and has a distinct odor (Fig. 3a). The clear appearance of the nanoemulsion was supported by a transmittance of 96.6±0.378%. The transmittance percentage close to 100% indicated that the preparation had attained nanoscale size with a clear and translucent look [27]. These findings were supported by particle size results, which have values of 17.8±0.1 nm. The small size of nanoemulsion correlates to higher penetration to skin via the transepidemal and transfollicular pathways [28].

Figure 3b depicts the organoleptic of the nanoemulgel produced by mixing the optimum nanoemulsion formula with the gel base. The visual of nanoemulgel is the same as that of nanoemulsion preparations, despite having a higher viscosity.

The PDI of the optimum RBO nanoemulsion formula was 0.51±0.00. Previous research explained that the PI value less than 0.7 indicated the particle size distribution that was well dispersed in the nanoemulsion system [29]. Low PDI value indicates higher the uniformity of globule size in nanoemulsion preparations [28].

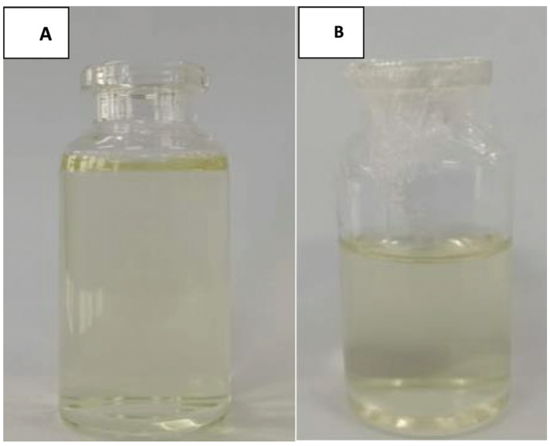
Zeta potential of the optimum RBO nanoemulsion formula was −3.56±0.70. Stable nanoemulsion results from a previous work had a similar zeta potential, namely 3.70±0.36 [30]. Negative values is thought to be influenced by the adsorption of −OH groups from the water phase to the interface [31].

The pH value of the optimal RBO nanoemulsion were 6.19±0.05. RBO nanoemulsion pH are within the range considered safe for topical preparations, which is between 5.5 and 6.5. This suitable pH condition ensures that the preparation is nonirritating to the skin [32].

Stability test

Room temperature and freeze-thaw stability tests revealed no changes in the organoleptic quality or

Figure 3



Organoleptic of RBO nanoemulsion (a) and nanoemulgel (b).

homogeneity of the optimal RBO nanoemulsion and nanoemulgel formulas. In terms of phase separation, color, clarity, and scent, the RBO nanoemulsion and nanoemulgel were unaltered. These results demonstrate the stability of the nanoemulsion and nanoemulgel throughout time.

In-vivo skin hydration and viscoelasticity study

The results of the irritation test showed that RBO nanoemulgel did not cause itching, redness, and swelling of the skin of 12 volunteers. This shows that RBO nanoemulgel is nonirritating and safe to use on human skin.

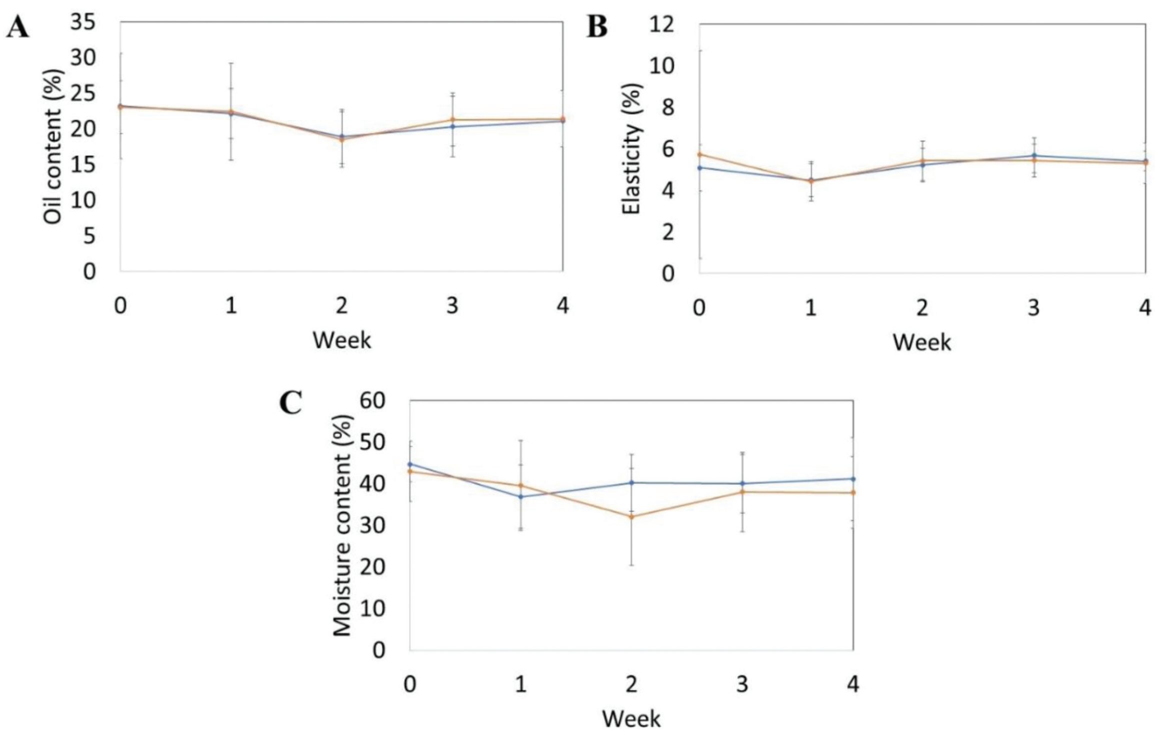
Moisture test on the parameters of water content, oil content, and elasticity in 12 volunteers is shown in Fig. 4. The effectiveness of the RBO nanoemulgel was compared between before and after results and between RBO-nanoemulgel and empty preparations. RBO-nanoemulgel shows no significant difference to the empty base on oil and elasticity parameter for 4 weeks of use ($P>0.05$) (Fig. 4a and b). However, there was significant in the use of the preparation every week ($P<0.05$) (Fig. 4a and b).

The effects of the treatment could not be significant due to short-term use and individual variances; thus, a longer test period and more volunteers are required [33]. Naeimifar *et al.* [34] explained that there was a significant improvement in skin firmness and tiring effect after applying an anti-wrinkle cream containing saffron extract and avocado oil for 12 weeks.

Solar UV-B harms skin by inducing morphological changes and skin aging, which is characterized by wrinkles, dryness, roughness, and hyperpigmentation as well as a loss of skin suppleness [35,36]. UV-B light absorption, which mostly took place in the epidermis, resulted in the production of ROS and transcription factors such AP-1 and NF-kB. These factors hinder collagen formation and increase the production of matrix metalloproteinases (MMPs) [37]. MMP-1 overproduction breaks down collagen fibrils, accelerating the development of wrinkles. In turn, damaged skin leads to increased ROS, which impairs collagen and ultimately results in rougher skin [36].

Plant extracts are added to antiaging formulations as ROS scavengers. Rice bran is known to contain high antioxidant compounds, such as tocopherols, tocotrienols, flavonoids, phenols, polyphenols, and squalene [5–7]. Oryzanol has strong antioxidant activity in scavenging free radicals, which allows it to

Figure 4



Results of oil content (a); elasticity (b); and moisture content (c) of 12 volunteers after application of RBO nanoemulgel (blue line) and nanoemulgel base (orange line).

improve skin texture by reducing oxidative damage and neutralizing ROS. In our study, the skin moisture in the RBO nanoemulgel group is higher than base group after 4 weeks treatment (Fig. 4c), although the difference was not statistically significant ($P>0.05$). The water in the skin rises as a result of the ability of antioxidants to protect it from oxidative damage. This could be caused by the increase in collagen formation, thereby improving skin structure and moisture content by reducing water loss. Previous research explained that tocopherol increased collagen production by downregulating MMP-1 [38].

Other studies suggest that antioxidant molecules help nourish skin cells and prevent pore blockage while the skin regenerates and the aging process is halted [39]. Previous studies have shown that nanoformulations containing RBO are efficient at boosting skin moisture [40,41].

This increase in effectiveness is also due to the nanoemulsion system in the preparation. The oil phase and the water phase in the nanoemulsion can interact with the lipid components in the skin [41]. The water phase in the nanoemulsion can increase the hydration of the outermost layer of skin, resulting in improved permeability, delivery, constant diffusion, and penetration of active ingredients [38]. Other studies have demonstrated that upon skin contact. Nanoemulgel released oily droplets from the gel network, which then permeated the stratum corneum of the skin and delivered drugs or active ingredients directly to the site of action [39].

Conclusion

The present findings suggest the possibility of using rice bran by-products as anti-aging which is acceptable to consumers. Nanosized nanoemulsion containing RBO were successfully optimized by Box–Behnken design as a homogenous, high-transmittance, clear solution with a pH range acceptable for skin. The optimized nanoemulsion and nanoemulgel was stable during the period of study and stability study. The optimized preparation did not show any irritation after the application on volunteer skin, proven its tolerability. The clinical skin ageing studies suggested that RBO nanoemulgel have superior increase of moisture content, compared with the base, after 4 weeks of application on the skin volunteer. In conclusion, our work opens a new platform which is promising for the development of RBO as a topical delivery strategy for management of aging.

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Nil.

Conflicts of interest
There are no conflicts of interest.

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