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Optimization of quercetin and titanium dioxide (TiO_2) concentrations as sunscreen agents in lip cream formulations

Optymalizacja stężeń kwercetyny i dwutlenku tytanu (TiO_2) jako substancji ochronnych przed promieniowaniem słonecznym w kremach do ust

ABSTRACT

Exposure of the body to ultraviolet radiation can cause negative effects, including sunburn and cancer. The lips are a particularly sensitive area and should be given special sun protection.

This study aimed to determine the optimal concentration of quercetin and titanium dioxide in lip creams and to assess their impact on physicochemical properties (pH, viscosity, lubricity) and the effectiveness of in vitro sun protection.

The results showed that quercetin and titanium dioxide had an impact on the physicochemical properties and in vitro sun protection effectiveness. The optimal formula obtained as a result of this research is a combination of quercetin and titanium dioxide at concentrations of 3.00% and 9.52%, respectively. The combination of these substances shows potential for use in creams that protect against the negative effects of ultraviolet radiation.

Keywords: quercetin, titanium dioxide, lip cream, ultraviolet radiation, sunscreen

STRESZCZENIE

Ekspozycja ciała na promienie ultrafioletowe może powodować negatywne skutki, między innymi oparzenia słoneczne oraz nowotwory. Szczególnie wrażliwym obszarem są usta, które powinny być objęte szczególną ochroną przeciwsłoneczną.

Celem badań było wyznaczenie optymalnego stężenia kwercetyny i ditlenku tytanu w kremach do ust oraz ocena ich wpływu na właściwości fizykochemiczne (pH, lepkość, smarowność) i skuteczność ochrony przeciwsłonecznej w warunkach *in vitro*.

Wyniki badań wykazały, że kwercetyna i ditlenek tytanu miały wpływ na właściwości fizykochemiczne i skuteczność ochrony przeciwsłonecznej *in vitro*. Optymalna formuła uzyskana w wyniku tych badań to połączenie kwercetyny i ditlenku tytanu o stężeniach odpowiednio 3,00% i 9,52%. Połączenie tych substancji wykazuje potencjał do zastosowania ich w kremach chroniących przed negatywnymi skutkami promieniowania ultrafioletowego.

Słowa kluczowe: kwercetyna, ditlenek tytanu, krem do ust, poromieniowanie ultrafioletowe, filtr przeciwsłoneczny

INTRODUCTION

Sunlight that reaches The Earth consist of ultraviolet radiation (UV): UVA (320-400 nm), UVB (290-320 nm) and UVC (100-280 nm). One of the positive effects of UV radiation on humans is that it stimulates the production of previtamin D [1, 2]. Additionally, erythema and pigmentation are two negative consequences of UV radiation on the skin [3]. Lip skin has a thinner stratum corneum and less melanin than skin on other regions of the body, making it more vulnerable to the harmful effects of UV radiation [4]. In the long term, UV radiation can cause squamous cell carcinoma, which is the most common type of lip cancer with a prevalence of more than 90% compared to other types of lip cancer [5]. Therefore, lip skin needs special defense against UV rays.

Protection against UV radiation on the lips can be provided by using sunscreen in the form of lip cream. Active sunscreen compounds that can be used in formulation are classified into chemical absorbers and physical blockers. One example of a chemical absorber compound is quercetin, while a physical blocker compound is titanium dioxide (TiO_2). Based on research conducted by Donglikar et al, quercetin in a concentration of 3% is able to provide protection from UV rays with a sun protection factor (SPF) value of 16.11 [6]. Unfortunately, quercetin's color is unattractive (yellow), so increasing the concentration of quercetin will reduce its acceptability [7]. In addition, titanium dioxide has a high refractive index, so it can be used as a white pigment [8]. Thus, in this study, quercetin will be combined with titanium dioxide to obtain an optimal sunscreen lip cream.

Sunscreen lip cream preparation was formulated in the form of water-in-oil (W/O) cream [9]. The W/O type emulsion was selected since it can adhere and shield the lip skin for a longer period of time because it doesn't readily fade when exposed to water. The combination of quercetin and titanium dioxide in the right proportions is expected to produce a sunscreen lip cream with acceptable physicochemical properties and sunscreen effectiveness. Therefore, in this study, the concentrations of quercetin and titanium dioxide were optimized using a factorial design to obtain the best lip cream formula. The effects of quercetin, titanium dioxide, and their combination on the physicochemical properties (pH, viscosity, and spreadability) were evaluated. In addition, the *in vitro* effectiveness of the sunscreen formulation were measured based on SPF, erythemal transmission (%TE), and pigmentation transmission (%TP).

MATERIALS AND METHODS

Materials

The materials used were quercetin (Tokyo Chemical Industry), titanium dioxide (Sigma Aldrich), cera alba (Sigma Aldrich), petrolatum (Sigma Aldrich), cetyl alcohol (Sigma Aldrich), sorbitan oleate (Sigma Aldrich), kaolin (Sigma Aldrich), glycerin (Sigma Aldrich), propylene glycol (Sigma Aldrich), Olea europaea fruit oil (Sigma Aldrich), alcohol 96% (Merck), water (PT. Brataco), pigment red 68 (Sigma Aldrich), isopropanol (Sigma Aldrich), and alcohol 90% (Merck). All materials used were pharmaceutical grade.

Formulation of Sunscreen Lip Cream

Lip cream formulas were designed as in table 1. The process began with weighing all ingredients according to the amounts required in the preparation using an analytical balance (Adventur, OHAUS). Then, cera alba, petrolatum, cetyl alcohol and sorbitan oleate were melted on waterbath (Memmert) at 70°C. After completely melting, the heat mortar was prepared, and the *Olea europaea* fruit oil (olive oil) was placed in a mortar. The melted mass was added and stirred until homogeneous mixture. Then kaolin and titanium dioxide were added to the mortar and stired to obtain homogeneous preparation. Water

Table 1 Formula of sunscreen lip creams containing quercetin and titanium dioxide. F(1): formula with low concentrations of quercetin and titanium dioxide, F(a): formula with high concentration of quercetin and low concentration of titanium dioxide, F(b): formula with low concentration of quercetin and high concentration of titanium dioxide, F(ab): formula with high concentrations of quercetin and titanium dioxide

Ingredients	Function	F(1)	F(a)	F(b)	F(ab)
Quercetin	Anti UV	0.5%	3.0%	0.5%	3.0%
Titanium dioxide	Anti UV	5.0%	5.0%	15.0%	15.0%
Cera alba	Oil phase	4.0%	4.0%	4.0%	4.0%
Petrolatum	Oil phase and emollient	10.0%	10.0%	10.0%	10.0%
Cetyl alcohol	Emulsifying and thickening agent	2.0%	2.0%	2.0%	2.0%
Sorbitan oleate	Emulsifying agent	10.0%	10.0%	10.0%	10.0%
Kaolin	Protective coating	9.0%	9.0%	9.0%	9.0%
Glycerin	Cosolvent	5.0%	5.0%	5.0%	5.0%
Propylene glycol	Cosolvent	3.0%	3.0%	3.0%	3.0%
Alcohol	Solvent of quercetin	15.0%	15.0%	15.0%	15.0%
Water	Water phase	20.5%	18.0%	10.5%	8.0%
Olea europaea fruit oil	Oil phase	15.0%	15.0%	15.0%	15.0%
Pigment red 68	Dye	1.0%	1.0%	1.0%	1.0%

Source: Own elaboration

at 70°C, heated on the hotplate (Thermo Scientific) was added. After the mortar temperature decreases, quercetin which had been dissolved in alcohol, glycerin and propylene glycol, were added. Pigment red 68 was then added into the mortar and stirred until homogeneous cream.

Evaluation of the Physicochemical Properties of the Sunscreen Lip Cream

Organoleptic Test

Organoleptic test was carried out visually by observing the lip cream preparation, including its color, texture and smell.

Homogeneity Test

Homogeneity test was performed by placing a 1-gram sample of the lip cream between two glass plates for visual observation. The lip cream was considered homogeneous if it exhibited a uniform consistency and evenly distributed color.

Emulsion Type Test

A drop of methylene blue was added to a tiny amount of lip cream on a glass plate, and the mixture was stirred until it was homogenous to verify the emulsion type. Subsequently examined under a microscope (CX33 Trinocular Microscope Olympus) [10]. The lack of uniform dispersion of methylene blue, observed as localized spots within the formulation, indicated that the aqueous phase was the internal phase, thereby confirming that the emulsion was the water-in-oil (w/o) type.

Spreadability Test

Spreadability test was performed by applying lip cream on the back of the hand five times, followed by visual observation [11]. The lip cream exhibited good smearability, as indicated by the color sticking abundantly and evenly on the skin.

pH Test

The preparation was put in a beaker to measure the pH. The pH meter (Denver), which has been calibrated, was placed inside the beaker and watched for a steady reading. The pH value was between 4-6. The pH value of the preparations was adjusted to the stable pH of quercetin (1-6) and the physiological pH of the lips (4-6.5) [11, 12].

Viscosity Test

Viscosity testing was accomplished by a VT-04 Viscometer (Rion). The test was carried out by placing a beaker containing the lip cream preparation, then installing the spindle and running the rotor.

Spreadability Test

The spreadability testing was was performed by placing 1 gram of the lip cream in the middle of the extensometer. Next, after 1 minute, loads ranging from 50 g to 250 g were applied every 1 minute. The diameter formed was measured [13].

Evaluation of In Vitro Effectivity of Sunscreen Lip Cream

In Vitro SPF Value Test

SPF value test was carried out by dissolving an amount of preparation equivalent to 2 mg of active sunscreen ingredient (combination of quercetin and titanium dioxide) in 10 ml of 90% alcohol. Then, 0.5 ml of the solution was diluted to 10 ml. Next, the absorption of the solution was measured using a UV-Vis spectrophotometer (Genesys 10S UV-VIS) at a wavelength of 290 nm until the wavelength showed an absorption of 0.05 (observation interval 1 nm). The SPF value was calculated using the equation below [14]:

$$Log\ SPF = \frac{AUC}{\lambda n - \lambda 1}$$

AUC = area under the curve
λn = the longest wavelength above 290 nm, which has an absorption value higher than 0.05
$\lambda 1$ = smallest wavelength (290 nm)

%TE and %TP Test

Determination of the %TE and %TP values was achieved by dissolving an amount equivalent to 2 mg of the active sunscreen ingredient (combination of quercetin and titanium dioxide) in 10 ml of isopropanol. Next, 0.5 ml of the solution was diluted to 10 ml. Then the absorption of the solution was measured using a spectrophotometer at wavelengths of 292.5-337.5 nm (%TP) and 322.5-372.5 nm (%TE) with intervals of 5 nm [15]. %TE and %TP were calculated using the equation below [16]:

$$\%TE = \frac{\Sigma(T.Fe)}{\Sigma Fe} \qquad \%TP = \frac{\Sigma(T.Fp)}{\Sigma Fp}$$

T = transmission percent value
Fe = erythema flux constanta
Fp = pigmentation flux constanta
ΣFe = total UV erythema flux
ΣFp = total UV pigmentation flux

Optimal Formula Determination and Verification

The optimal formula for sunscreen lip cream was determined from the analysis results of the Design Expert 13.0 Software. The selected formula was the one that had a desirability value close to 1 with response parameters pH, viscosity, spreadability, SPF value, %TE and %TP. Next, the optimal formula was verified by testing three repetitions to compare the adequacy of the predicted response of the Design Expert 13.0 Software with the observation results. Before statistical analysis, the data were tested for normality. A one-sample t-test was conducted at a 95% confidence level. The data were not significantly different if the p-value was > 0.05.

RESULTS

Physicochemical Properties of the Sunscreen Lip Cream

Organoleptic properties

The formulations of sunscreen lip cream were pink and coral, soft texture and were odourless (fig. 1).

Homogeneity

The results of the homogeneity test demonstrated that sunscreen lip cream had a homogeneous consistency and the color was evenly distributed (fig 2).

Emulsion Type

The results of the emulsion type test indicated the water-in-oil (W/O) emulsion (fig. 3).

Spreadability Test

The result of the test showed good spreadability as indicated by the color sticking abundantly and evenly on the skin (fig. 4).

pH Value

Based on Table 2, the results of the pH testing of the sunscreen lip cream preparations showed that F(1) and F(a) met the pH value requirements, while F(b) and F(ab) did not fulfilled the prerequisites, which was in the range of 4-6.

Viscosity

The results of the viscosity testing of the preparation met the requirements range of 100-200 dPas, which is presented in table 2. These results are also showed in the contour plot (fig. 5).

Spreadability

The findings demonstrated that the concentration of titanium dioxide and quercetin significantly reduced the spreadability of the preparations. The results are presented in table 2 and in the contour plot (fig. 5).

Table 2 Viscosity, spreadability and pH value of sunscreen lip creams containing quercetin and titanium dioxide

Formulation	pH value*	Viscosity (dPa.s)*	Spreadability (cm/gram)*
F(1)	5.2367±0.0499	111.67±6.2361	6.80±0.3266
F(a)	5.5167±0.0287	126.67±6.2361	6.20±0.2944
F(b)	6.1667±0.0499	163.33±2.3570	5.40±0.1633
F(ab)	6.5867±0.0464	181.67±6.2361	5.17±0.1247

Data are presented as mean ± standard deviation (n=3)
*Test response included in a factorial design

Source: Own elaboration

Fig. 1 Organoleptic properties of sunscreen lip cream formulations. F(1): 0.5% quercetin and 5% titanium dioxide, F(a): 3% quercetin and 5% titanium dioxide, F(b): 0.5% quercetin and 15% titanium dioxide, F(ab): 3% quercetin and 15% titanium dioxide. **Source:** Own elaboration

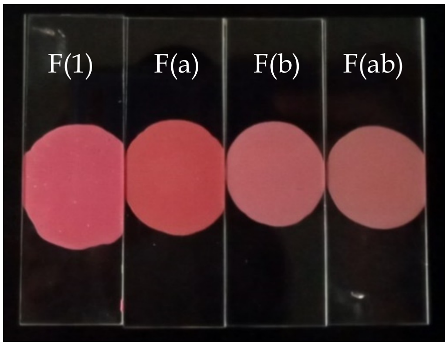

Fig. 2 The result of the homogeneity test of sunscreen lip cream formulations. F(1): 0.5% quercetin and 5% titanium dioxide, F(a): 3% quercetin and 5% titanium dioxide, F(b): 0.5% quercetin and 15% titanium dioxide, F(ab): 3% quercetin and 15% titanium dioxide
Source: Own elaboration

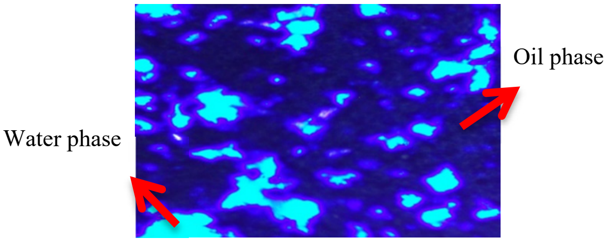

Fig. 3 The result of evaluation of water-in-oil (W/O) emulsion type of sunscreen lip creams containing quercetin and titanium dioxide **Source:** Own elaboration

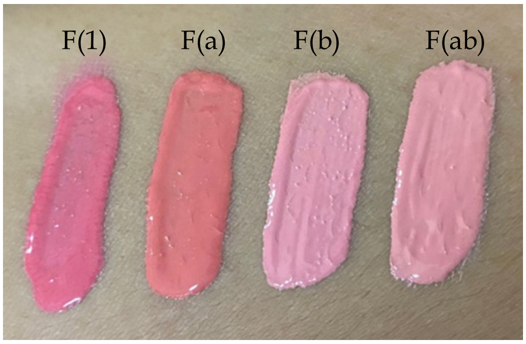

Fig. 4 The result of spreadability testing of sunscreen lip cream formulations. F(1): 0.5% quercetin and 5% titanium dioxide, F(a): 3% quercetin and 5% titanium dioxide, F(b): 0.5% quercetin and 15% titanium dioxide, F(ab): 3% quercetin and 15% titanium dioxide
Source: Own elaboration

Table 3 SPF *in vitro* of sunscreen lip cream with quercetin and titanium dioxide

Formulation	SPF *(Sun Protection Factor)**	
F(1)	14.32±0.3876	Maximum
F(a)	18.86±0.4766	Ultra
F(b)	20.44±0.4710	Ultra
F(ab)	26.09±0.4138	Ultra

Data are presented as mean ± standard deviation (n=3)
*Test response included in a factorial design

Source: Own elaboration

Table 4 %TE and %TP value of sunscreen lip cream with quercetin and titanium dioxide

Formulation	%TE*	%TP*	Sunscreen Category
F(1)	0.3197±0.00645	0.2412±0.00068	Total block
F(a)	0.1897±0.00830	0.1389±0.00651	Total block
F(b)	0.0293±0.00503	0.0197±0.00342	Total block
F(ab)	0.0011±0.00004	0.0004±0.00001	Total block

Data are presented as mean ± standard deviation (n=3)
*Test response included in a factorial design

Source: Own elaboration

In Vitro Effectivity of Sunscreen Lip Cream

SPF Value

SPF test *in vitro* results are collected in Table 3. The results showed that increasing concentration of quercetin and titanium dioxide had a significant effect on elevating the SPF value. These results are presented in the contour plot (fig. 5).

%TE and %TP Values

The results of the %TE and %TP evaluation are in Table 4. All formulas had met the requirements, namely <1. These results are presented in the contour plot (fig. 5).

Optimal Formula Determination and Verification Results

The optimal formula of sunscreen lip cream was determined from the results of the overlay plot analysis (fig. 6). The result of verifying the optimal formula with pH, viscosity, spreadability, SPF value, %TE and %TP through the one sample t-test showed a p-value > 0.05, which is presented in Table 5.

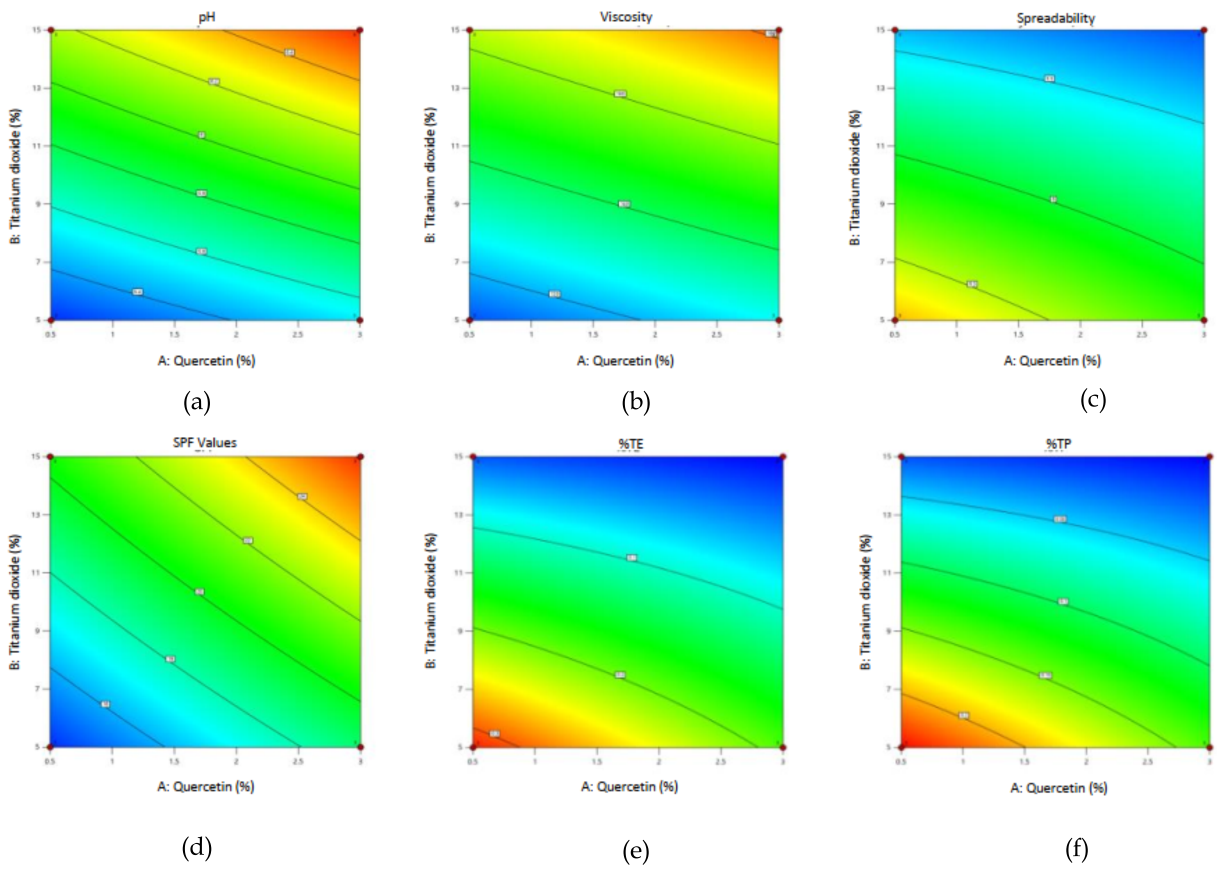

Fig. 5 Contour plot of response: (a) pH, (b) viscosity, (c) spreadability, (d) SPF values, (e) %TE values and (f) %TP values. **Source:** Own elaboration

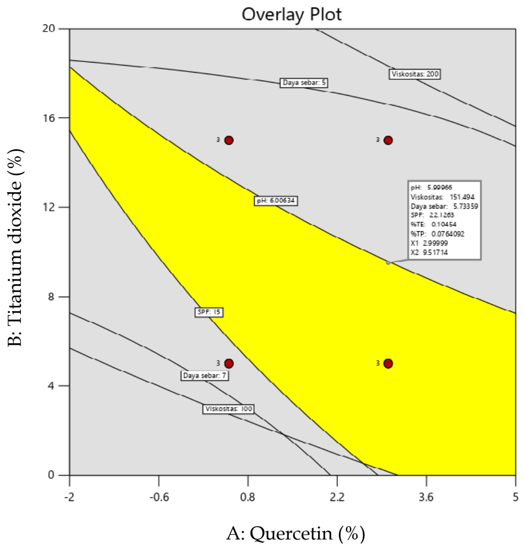

Fig. 6 Overlay plot of the optimal formula **Source:** Own elaboration

Table 5 Verification result of optimal formula of sunscreen lip cream containing quercetin and titanium dioxide

Response	Prediction result	Observation result	*p-value*
pH	6.00	5.84	0.500
Viscosity (d.Pas)	151.511	151.667	0.496
Spreadability (cm/gram)	5.73	5.83	0.500
SPF	22.129	21.69	0.499
%TE	0.104	0.106	0.500
%TP	0.076	0.045	0.500

Source: Own elaboration

DISCUSSION

Physicochemical properties of the sunscreen lip creams showed that increasing the concentration of quercetin in the preparation resulted in yellow color, so that when mixed with red dye, coral color was obtained. Meanwhile, increasing the concentration of titanium dioxide could give a more opaque impression. Sunscreen lip creams had a homogeneous consistency, were w/o emulsion, with good spreadability.

The results of pH value evaluation showed that increasing the concentration of quercetin and titanium dioxide had a significant effect on elevating the pH value. These results are presented in the contour plot (fig. 5). Quercetin did not directly cause an increase in pH, rather, the pH environment influences the protonation state of its hydroxyl groups, particularly the 3-OH group. At pH 4–6, quercetin predominantly exists in its ionized form (e.g., H_4A^-), where it can interact with available protons (H^+), leading to changes in the ionic balance of the formulation [17]. Titanium dioxide could increase the pH value due to alkaline pH 8 in suspension form [18].

The results of the viscosity testing showed that the concentration of quercetin and titanium dioxide had a significant effect on increasing the viscosity of the preparations. The amount of water was reduced to offset the increase in quercetin and titanium dioxide content. The preparations became more viscous as a result.

In spreadability testing, increasing the concentration of quercetin and titanium dioxide reduced the spreadability. This feature is related to the viscosity, the higher viscosity value, the lower spreadability, but still meeting the requirements in the range of 5-7 cm after being given a load of 250 gram. The results showed that the concentration of quercetin and titanium dioxide had a significant effect on reducing the spreadability of the preparation.

In vitro effectivity of sunscreen lip creams showed that quercetin could increase the SPF value because of the chromophore group, which can absorb UV radiation [19]. Meanwhile, the titanium dioxide, a physical UV blocker, can protect from UV rays, primarily through the absorption of UV radiation by utilizing its energy band gap. In addition, TiO_2 is also known to protect against UV through a combination of absorption, reflection, and scattering mechanisms [20]. These results are in accordance with research conducted by Choquenet et al, which stated that the combination of quercetin and titanium dioxide yielded higher SPF values than when used separately [21]. However, further studies are needed to determine whether this effect is synergistic or merely additive.

Erythema transmission percent (%TE) is the sum of sunlight that is passed through after hitting the sunscreen and causes skin erythema (reddish skin). Pigmentation transmission percent (%TP) describes the amount of sunlight that is passed on after sunscreen is applied, so it can cause skin pigmentation (darker skin) [22]. The results showed that the concentration of quercetin and titanium dioxide had a significant effect on decreasing the %TE and %TP values, while the combination of these two ingredients had a significant effect on increasing the %TE and %TP values. İt results from quercetin and titanium dioxide's ability to absorb UV A and UV B rays, which cause pigmentation and erythema, so increasing the concentration of quercetin and titanium dioxide can reduce %TE and %TP values [2, 24]. The lower the %TE and %TP value, the better the ability of sunscreen preparations to protect the skin from erythema and pigmentation [23].

The optimal formula for sunscreen lip cream contained 3.00% quercetin and 9.517% titanium dioxide with a desirability value of 0.915. The estimated features were: pH 6; viscosity 151.511 dPas; spreadability 5.733 cm; SPF 22.129; %TE 0.104; and %TP 0.076. The results of optimal formula verification showed that Design Expert 13.0 Software could make response prediction correctly, and there was no significant difference between the prediction result and the observation result.

CONCLUSIONS

To conclude, the increase in the concentration of quercetin elevated the pH value, reduce the viscosity and the spreadability of the preparation. Increasing the concentration of quercetin and titanium dioxide can also raise the SPF and reduce the %TE and %TP values. The combination of quercetin and titanium dioxide can increase the %TE and %TP values. The combination concentration of quercetin and titanium dioxide (TiO_2), which shows optimal physicochemical properties and *in vitro* sunscreen effectiveness in sunscreen lip cream preparations, is 3.00% quercetin and 9.517% titanium dioxide.

These findings suggest potential applications in cosmetic chemistry, particularly in the formulation of multifunctional natural-based sunscreens. In cosmetology, the optimized lip cream may help create products that give photoprotection in addition to aesthetic advantages, demonstrating its usefulness in the creation of protective and health-conscious cosmetics.

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CONFLICT OF INTEREST

The authors declare no conflict of interests.

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