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Original Article

STATISTICAL OPTIMIZATION AND STABILITY STUDY OF QUERCETIN-LOADED MICROEMULSION

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ABSTRACT

Objective: This research aims to develop a quercetin microemulsion system to improve the solubility of quercetin and to study the stability of the microemulsions.

Methods: The microemulsion is prepared by water titration method using isopropyl myristate (oil), Tween 60®/Span 80® (3:2) (surfactant) and ethanol (co-surfactant). Two different aqueous phases, water or NaCl solution, were used to prepare microemulsions and the influence of each parameter was described. DPPH scavenging and anti-tyrosinase activity were performed along with chemical stability to evaluate the functional stability of microemulsions.

Results: The influence of percentage of oil phase (variable A) on the solubility of quercetin was less significant than that of percentage of surfactant/co-surfactant (variable B). Compared to those prepared with water (variable C), the solubility of quercetin in microemulsions prepared with NaCl solution significantly increased. The ratio of the high level to low level for solubility of three variables was 1.135, 1.315 and 1.591 respectively. Increasing variable A and B led to an increase in the particle size of microemulsions from 120.08 nm to 188.38 nm and 48.18 nm to 260.28 nm, respectively. The influence of variable B was quite significant, while variable C has no significant effect on particle size. Quercetin microemulsions showed good chemical and functional stability when stored at 4 °C. Under other conditions, especially at 40 °C, the activity of the microemulsion is considerably reduced.

Conclusion: The influence of different variables on the characteristics of microemulsions was complicated. Care must be taken in the composition and storage conditions of these formulations.

Keywords: Quercetin, Microemulsion, Antioxidative, Anti-tyrosinase

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INTRODUCTION

With the development of the aging society, the anti-aging of the skin has always been a topic that has attracted attention. Many studies have pointed out that the clinical signs of aging are mainly affected by external factors, especially ultraviolet (UV) exposure. Chronic UV radiation exposure from the sun has been shown to cause premature skin aging (photoaging) and skin cancer; while acute exposure may cause sunburn, immune suppression, and connective tissue degradation [1, 2]. These skin damages due to ultraviolet radiation (UVR) exposure is the result of the generation of free radicals and skin inflammation [3]. Although the physical or chemical sunscreens can reduce skin damage caused by UVR, their protective effects are not satisfactory due to inadequate use, lack of antioxidative and anti-inflammatory properties, incomplete spectral protection and toxicity [4]. A new concept in cosmetics and pharmaceutical research and development is to use substances that can protect the skin from UVR damage and may also have biological UV filtering activity, which is the so-called "biological filters [5]. Some naturally occurring compounds, such as quercetin, have been proven to exhibit several biological properties, including anti-inflammatory, antiviral, and antimicrobial effects [6, 7]. In addition, quercetin not only has a sun protection effect by absorbing ultraviolet radiation [8], but also has a whitening effect on the skin by inhibiting tyrosinase [9]. It is also a good antioxidant and has rejuvenated activity on terminally senescent cells [10]. Therefore, the topical preparation of quercetin has quite excellent development potential.

However, the low solubility and low skin penetration ability of quercetin limit its application. Different methods have been studied for the skin delivery of quercetin [11-13]. Unfortunately, insufficient drug loading, large droplet size, and poor stability is often the disadvantages of those carrier systems [14-17]. Microemulsions has become an attractive technique for the percutaneous administration of drugs due to high solubilizing capacity and thermodynamic stability [18, 19]. Studies have pointed out that the incorporation of quercetin into microemulsions can obtain a more optimized effect than that of emulsions [20]. However, microemulsion systems are diverse in composition; different pharmaceutical properties such as type of microemulsions, the particle size of the dispersion phase, stability are influenced by complex process conditions. Detailed research is necessary in order to apply these systems to "cosmetic or pharmaceutical preparations" [21]. Experimental designs may obtain the most information from the fewest experiments [22, 23], but there were very few studies on the effect of the composition on the properties of quercetin-loaded microemulsion by experimental design. Therefore, a two-level factorial experimental design was utilized in this study to investigate the influence of the composition variables on the production of quercetin-loaded microemulsions, and the chemical and functional activity studies of microemulsions were also carried out to evaluate the stability of quercetin microemulsions.

MATERIALS AND METHODS

Materials

Quercetin, 1,1-diphenyl-2-pycrylhydrazyl (DPPH), tyrosinase, isopropyl myristate (IPM) and phosphoric acid were purchased from Sigma-Aldrich (Merck KGaA, Darmstadt, Germany). Sorbitan monooleate (Span 80®), polyoxyethylene sorbitan monostearate (Tween 60®) and polyoxyethylene sorbitan monooleate (Tween 80®) were from Nippon Shinyaku Co., Ltd. (Kyoto, Japan). Liquid chromatography grade methanol was purchased from Macron Fine

Chemicals (Avantor Performance Materials, Center Valley, PA, USA). All other chemicals were of analytic grade.

Construction of pseudo-ternary phase diagrams

The existence zones of microemulsions were determined by constructing pseudo-ternary phase diagrams using the water titration method. Based on the results obtained, appropriate parameters were selected for further experimental design. In this study, IPM was selected as an oil phase, Tween 60®/Span 80® (3:2) and ethanol were selected as a surfactant and as co-surfactant, and they were firstly mixed at the ratio of 1:1 (w/w) to form the mixed surfactant (Smix). For the aqueous phase, two different aqueous phases, water or 150 mmol NaCl solution, were used to prepare microemulsions.

For each pseudo-ternary phase diagram, the oil mixtures were prepared with the weight ratio of oil to Smix at 9.5:0.5, 9:1, 8:2, 7:3, 6:4, 5:5, 4:6, 3:7, 2:8, 1:9, and 0.5:9.5, respectively. To these mixtures, water was added drop-wise and mixed thoroughly by magnetic stirrers at room temperature. Following the addition of each drop of water, the mixture was visually examined for transparency. The titration of water was stopped when the turbid mixtures were converted into a transparent solution. The amounts of water added in each group were recorded and marked on a pseudo ternary phase diagram with one axis representing the aqueous phase, the second representing oil, and the third representing Smix. Next, use 150 mmol NaCl solution instead of water for another comparison. On the basis of the pseudo ternary phase diagrams results, the appropriate composition ratio of the microemulsions will be selected for the experimental design study [24].

2^3 Full factorial experimental design

Preparation of microemulsions

During the preparation of microemulsions, different composition ratios will affect the performance of microemulsions. In order to determine the influence of manufacturing conditions on product characteristics and obtain the optimal manufacturing conditions, 2^3 full factorial experimental design was applied [25]. Three independent variables in this experiment, the percentage of oil phase (variable A), the percentage of Smix (variable B), the type of aqueous phase (variable C), were selected to investigate the influence on the solubility of quercetin and the particle size of the microemulsions. Each variable appears at two appropriate levels (low level (-1), high level (+1)), and the value of levels was selected based on the results obtained from the pseudo-ternary phase diagrams. The experimental variables and the design matrix are given in table 1 and table 2. For example, in the first experiment (ME-1) shown in table 2, the variables A, B, and C are all low levels; that is, the composition of the microemulsion is 28% of the oil phase, 62% of Smix and 10% of water. Eight different microemulsions were carried out in random order according to the preparation method described in the previous "Pseudo-Ternary Phase Diagram" section, and the properties (solubility and particle size) were tested.

Table 1: Two levels of three variables in 2^3 full factorial experimental design

Variables	Low level (-1)	High level (+1)
A: Percentage of oil phase	28%	31%
B: Percentage of Smix	62%	65%
C: Type of aqueous phase	water	NaCl solution

Table 2: A design matrix of 2^3 full factorial design

Formulation	Independent variables		
	A: Percentage of oil phase	B: Percentage of Smix	C: Type of aqueous phase
ME-1	-1	-1	-1
ME-2	+1	-1	-1
ME-3	-1	+1	-1
ME-4	+1	+1	-1
ME-5	-1	-1	+1
ME-6	+1	-1	+1
ME-7	-1	+1	+1
ME-8	+1	+1	+1

Solubility test

Solubility of quercetin in different microemulsions was determined by the shake flask method [26]. An excess of quercetin was added to a single vial containing 10 ml of the microemulsion. After sealing, the mixture was shaken in a water bath shaker kept at room temperature for 72 h. The mixture was centrifuged at 12,000 rpm for 5 min and then filtered through a 0.45 μm membrane filter. The filtrate was appropriately diluted and quantified by high-performance liquid chromatography **(**HPLC). HPLC analyses were performed using a Hitachi (Japan) liquid chromatograph equipped with a photodiode array detector. Samples were injected manually and the separation was performed in a C18 column with a mobile phase of methanol/water (60:40, v/v) containing 0.5% phosphoric acid and detected at 374 nm [27].

Particle size test

Mean particle size of different microemulsions were determined by were determined by using a dynamic light-scattering system [28]. Samples were not diluted and the measurements were performed at 25 °C.

Statistical analysis

All data were expressed as means±standard deviations (mean±SD). Statistically significant differences were identified using the student's t-test with $P<0.05$ as a minimal level of significance. Statistical evaluation of the effects of the factors on the solubility and particle size was performed applying one-way analysis of variance (ANOVA) at a 0.05 level, using a commercially available software package, Design Expert® V. 7.1.3 (Stat-Ease, USA).

***In vitro* release studies**

Depending on the results of the experimental design, appropriate microemulsions were selected for the *in vitro* release test. *In vitro* release studies were carried out using modified Franz diffusion cells with cellulosic membrane [17]. First, the cellulose membrane was fixed between two compartments of the cell (the donor and receptor compartment). The receptor medium was filled with 10 ml of pH 7.2 phosphate-buffered saline (PBS)/Tween 80® (9:1), and was constantly stirred by using of magnet stirring at 600 rpm and 37±1 °C. Microemulsion containing quercetin was placed in the donor part and covered to avoid water evaporation. At predetermined time periods (5 min, 10 min, 15 min, 20 min, 30 min, 1h, 3h, 6h, 12h and 24 h), 0.2 ml of sample was withdrawn from the receptor solution and replenished with 0.2 ml of freshly prepared buffer. Each sample was repeated in triplicate (n=3). Cumulative released drug ($W_{(n)}$) was calculated by the following equation [17]:

$$W_{(n)} = C_{(n)} \times V + V_i \times \sum [C_i]$$

Where $C_{(n)}$ is the concentration of the drug (mg/ml) determined at nth sampling interval, V is the individual Franz diffusion cell volume (ml), C_i is the concentration of drug for i^{th} sample (mg/ml), and V_i is the sampling volume (ml).

Dissolution release kinetics

To study the mechanism of drug release from different microemulsions, drug release data were analyzed according to the zero-order, first-order, and Higuchi model. The equations were described as follows [29, 30]:

Zero-order model: $Q_t - Q_0 = k_0 t$

First-order model: $\ln Q_t - \ln Q_0 = k_1 t$

Higuchi model: $Q_t = k_H t^{1/2}$

Where Q_t is the amount of drug released at time t, Q_0 is the amount of drug released at time t = 0, k_0 is the zero-order release constant, k_1 is the first-order release constant, k_H is the release constant of Higuchi.

Stability test of microemulsions

The optimal microemulsion formulations were stored at 4 °C (75% R. H.), 30 °C (75% R. H.), 40 °C (75% R. H.), respectively, for 3 mo. Chemical properties (the content of quercetin) and functional activity (DPPH free radical scavenging ability and inhibition of tyrosinase activity) were tested at 0, 1, 2, and 3 mo [31].

Chemical stability test

The microemulsion containing quercetin was diluted in methanol, and the mixture was centrifuged at 12,000 rpm for 5 min and then filtered through a 0.45 μm membrane filter. The filtrate was appropriately diluted and evaluated for quercetin content by HPLC method [27].

Functional stability test: DPPH free radical scavenging assay

The antioxidant activity of microemulsions was determined according to the modified method previously described by Fenglin [32]. The antioxidant activity was measured by the bleaching rate of DPPH. In its radical form, DPPH absorbs at the wavelength of 517 nm, but its absorption decreases upon reduction by an antioxidant or a radical compound. The microemulsion (150 μl) was added to 50 μl of a 0.1 mmol methanol DPPH solution. Methanol without the microemulsion was used as a control. The decrease in absorbance was determined at 517 nm after 30 min at room temperature in the dark. The decrease in the absorbance values of the samples was correlated with the control values and then the percentage of DPPH scavenging was calculated by the formula below [33]:

$$\% \text{ inhibition} = [(A_0 - A_i) / A_0] \times 100$$

Where A_0 and A_i denote the absorbance at 517 nm of the control and the test samples.

Functional stability test: tyrosinase inhibition assay

The tyrosinase inhibition assay was performed based on a previous study with slight modification [34]. Samples were prepared in a 96-well plate and the components were added as follows: 50 μl phosphate buffer (pH 6.8), 100 μl sample and 50 μl L-tyrosine solution. After incubation at 37 °C for 10 min, the reaction was initiated by adding 20 μl tyrosinase (350 U/ml) to each well and incubating at 37 °C for 30 min. The enzyme activity was determined by measuring the absorbance at 475 nm.

The percentage of tyrosinase inhibition was calculated as follows:

$$\text{Tyrosinase inhibition}(\%) = \{[(A - B) - (C - D)] / (A - B)\} \times 100$$

Where A is the absorbance of the control with the enzyme (L-tyrosine mixed with the enzyme in the buffer), B is the absorbance of the control without the enzyme (L-tyrosine in the buffer), C is the absorbance of the test sample with the enzyme (L-tyrosine mixed with the enzyme and test sample in the buffer) and D is the absorbance of the test sample without the enzyme (L-tyrosine mixed with the test sample in the buffer).

RESULTS AND DISCUSSION

Pseudo-ternary phase diagrams

Pseudo-ternary phase diagrams were constructed to determine the microemulsion region using the water titration method. This region, which is clear and homogenous, shows the range of possible concentrations of microemulsion components that can form a single-phase microemulsion with each other. Two pseudo-ternary phase diagrams constructed with different aqueous phases (water and NaCl solution) are shown in fig. 1A and fig. 1B respectively. It was obvious that by increasing the percentage of Smix, the microemulsion region in the phase diagram increased. In addition, by changing the water phase from water to NaCl solution, the microemulsion region area extended slightly.

2^3Full factorial experimental design

According to the 2^3 full factorial experimental design method, three kinds of independent variable were selected in this experiment, namely variable A (percentage of oil phase), variable B (percentage of Smix), and variable C (type of aqueous phase). There are two levels for each variable, namely high level [+1] and low level [-1]. Two levels of three variables were chosen from each pseudo-ternary phase diagram to ensure the achievement of microemulsions (table 3). The factorial design experiments were performed in random order, and the responses were measured as the solubility of quercetin and particle size. The design matrix and data obtained from a duplicate of the 2^3 experiments is given in table 3.

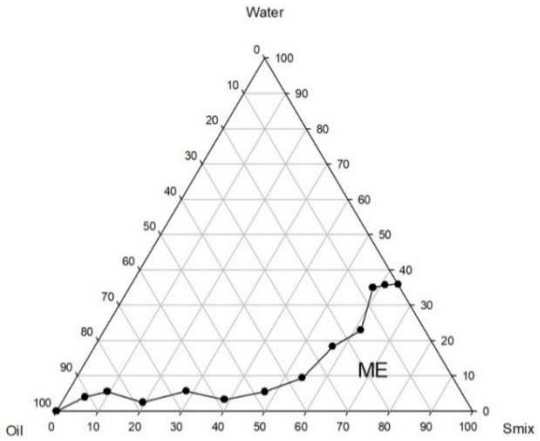
Table 3: Physical characteristics of different microemulsions

Random no. (Formulation)	Independent variables			Response *	
	A: Percentage of oil phase	B: Percentage of Smix	C: Type of aqueous phase	Solubility (mg/ml)	Particle size (nm)
1 (ME-3)	28%	65%	water	7.14±0.25	190.5±1.0
2 (ME-7)	28%	65%	NaCl solution	11.20±0.11	237.5±1.3
3 (ME-2)	31%	62%	water	6.15±0.18	41.5±0.8
4 (ME-5)	28%	62%	NaCl solution	8.20±0.06	31.9±0.7
5 (ME-4)	31%	65%	water	7.82±0.14	296.9±1.9
6 (ME-8)	31%	65%	NaCl solution	12.50±0.20	316.2±1.2
7 (ME-6)	31%	62%	NaCl solution	8.59±0.19	98.9±0.9
8 (ME-1)	28%	62%	water	5.23±0.15	20.4±0.6

* Values are expressed as mean±SD (n=3)

Among the eight prescriptions, the solubility of quercetin was between 5.23 and 12.50 mg/ml, which was compared with the solubility of quercetin in water (0.013 mg/ml) [27] and IPM (0.18 mg/ml) [17]. Microemulsions significantly improved the solubility of quercetin. On the other hand, the particle size distribution of the microemulsion ranged from 20.4 to 316.2 nm. These results were further analyzed in detail with Design Expert® software to find out the variables that have an influence on these properties. A half-normal plot is a plot of the value of effect estimates against their cumulative normal probabilities. The half-normal probability plot is

shown in fig. 2 and displays all significant factors affecting the quercetin solubility and particle size of microemulsions. All of the effects that lie along the line are negligible, whereas the significant effects lie far from the line. The important effects which influence the solubility of quercetin seem to be the main effects of A, B, C and the BC interaction which are far from the line (fig. 2A). The statistical differences were assessed using the ANOVA test to prove these results (table 4 and 5).



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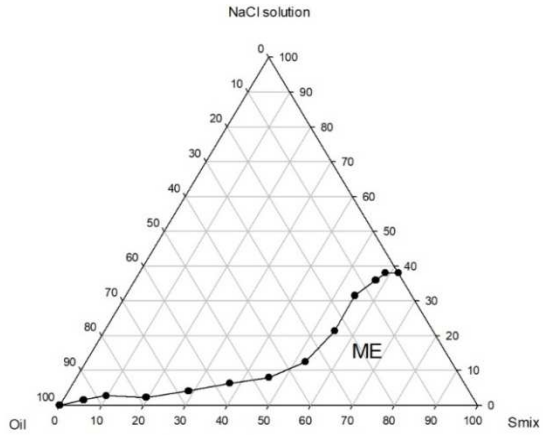


B

Fig. 1: Pseudo-ternary phase diagrams containing isopropyl myristate as an oily phase, tween 60®/Span 80® (3:2) (surfactant) and ethanol (cosurfactant) with smix ratio of 1:1 (A) water as an aqueous phase (B) NaCl solution as an aqueous phase

Table 4: Analysis of variance for solubility

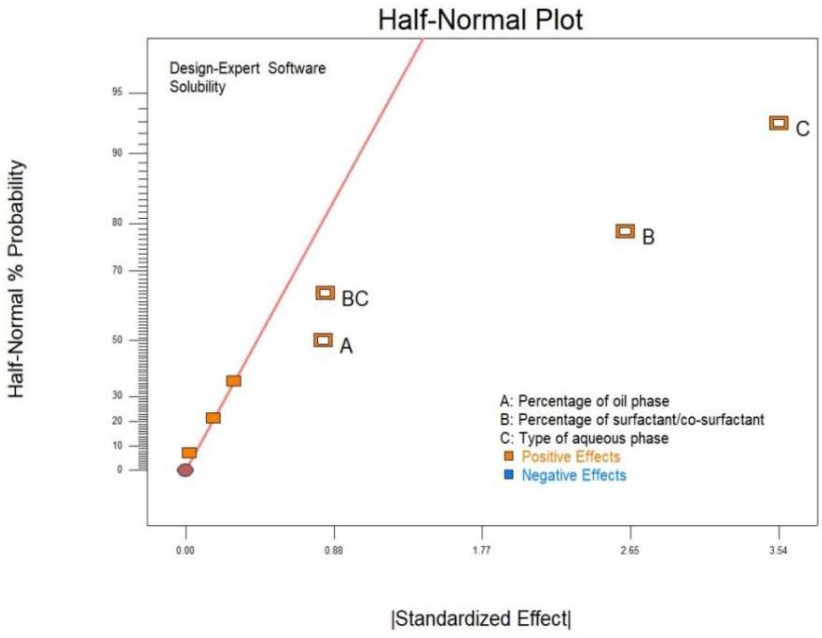
Source of variation	Degrees of freedom	Solubility			
		Sum of squares	Mean square	F	Prob > F *
Model	4	41.52	10.38	140.00	0.0010
A	1	1.35	1.35	18.25	0.0235
B	1	13.76	13.76	185.51	0.0009
C	1	25.03	25.03	337.55	0.0004
BC	1	1.39	1.39	18.69	0.0228
Residual	3	0.22	0.074		
Cor total	7	41.74			

* Significant: P < 0.05

Table 5: Analysis of variance for particle size

Source of variation	Degrees of freedom	Particle size			
		Sum of squares	Mean square	F	Prob > F *
Model	2	99302.60	49651.30	59.32	0.0003
A	1	9329.78	9329.78	11.16	0.0205
B	1	89972.82	89972.82	107.49	0.0001
Residual	5	4180.37	836.07		
Cor total	7	1.035E+005			

* Significant: P < 0.05



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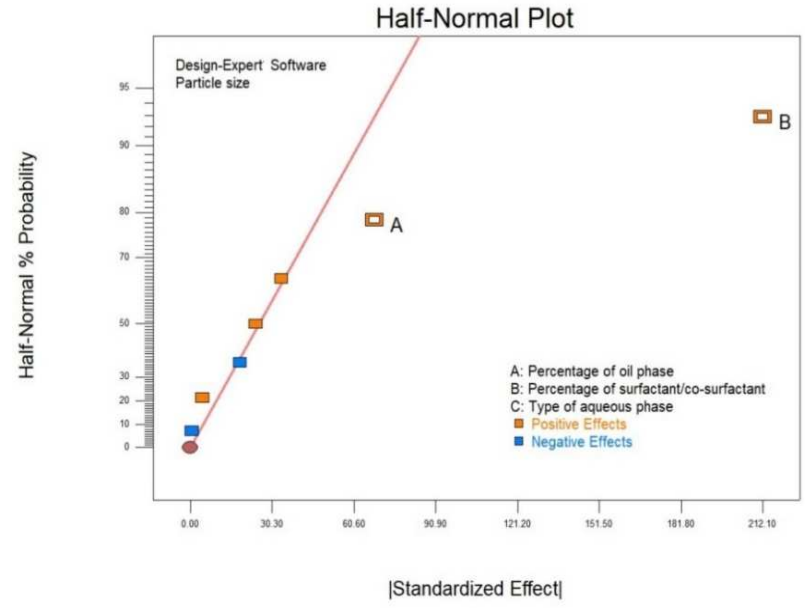
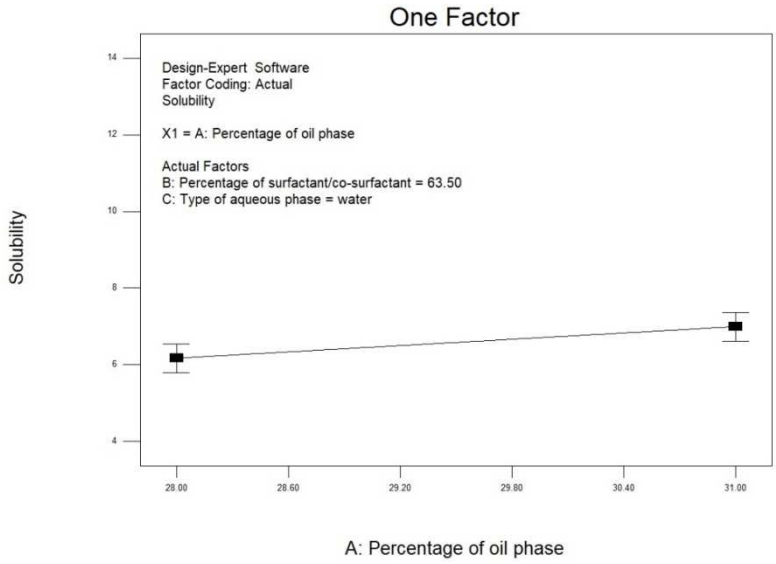


B

Fig. 2: The half-normal plot used for identifying all significant effects (A) the effect on quercetin solubility of microemulsions (B) the effect on the particle size of microemulsions

As shown in fig. 3A-D, the response value of the effects shows that all the effects of the three variables are positive. That is to say that increased variables A, B and C led to increasing quercetin solubility of microemulsions and exhibited the best results arising from the high level of three variables. The influence of variable A is less significant among the three variables. For instance, the solubility of quercetin of microemulsions at a low level of variable A was 6.17 mg/ml, compared to 7.00 mg/ml at a high level of variable A. The ratio of the high level to low level for solubility was 1.135. For variable B and variable C, the solubility of quercetin of microemulsions at a low level was 5.69 mg/ml and 6.36 mg/ml, compared to 7.48 mg/ml and 10.12 mg/ml, the ratio of the high level to low level for solubility was 1.315 and 1.591 respectively. Increasing the percentage of oil phase from low level to high level only caused a slight increase in the solubility of quercetin of microemulsions. Although increasing the percentage of oil phase has a positive effect on the solubility of quercetin in microemulsion, dissolving or dispersing of quercetin in the microemulsion was mainly done into the interfacial layer of emulsifying agents (surfactant and co-surfactant) and only small quercetin quantity was dispersed into oil phase. Therefore, the influence of variable A (percentage of oil phase) (fig. 3A) on the solubility of quercetin (fig. 3A) was not as significant as that of variable B (percentage of Smix) (fig. 3B). This result is consistent with the previous study by Kajbafvala [17]. As for variable C (type of aqueous phase), compared to those prepared with water (fig. 3E), the solubility of quercetin in microemulsions prepared with NaCl solution (fig. 3F) significantly increased. This result should be due to the fact that the solubility of quercetin in NaCl solution is greater than that in water [35].


One Factor
Design-Expert Software
Factor Coding: Actual
Solubility
X1 = A: Percentage of oil phase
Actual Factors
B: Percentage of surfactant/co-surfactant = 63.50
C: Type of aqueous phase = water
Solubility
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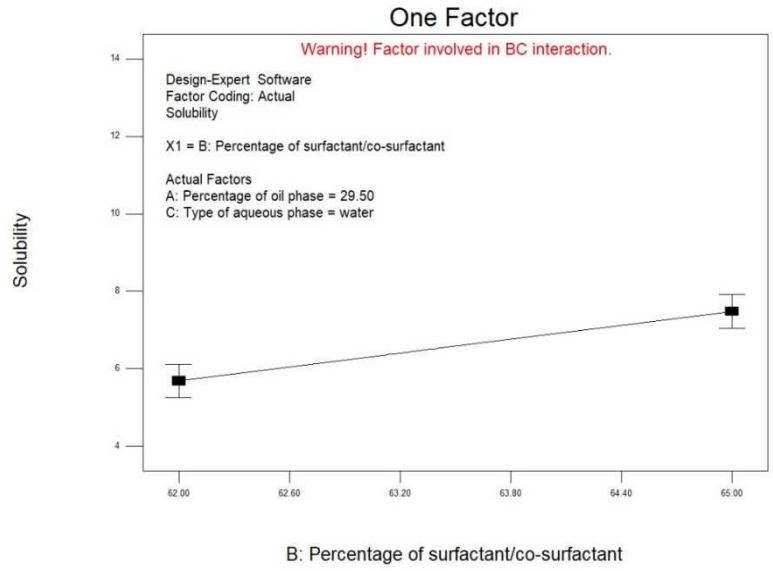
A: Percentage of oil phase

A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Factor Coding: Actual
Solubility
X1 = B: Percentage of surfactant/co-surfactant
Actual Factors
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C: Type of aqueous phase = water
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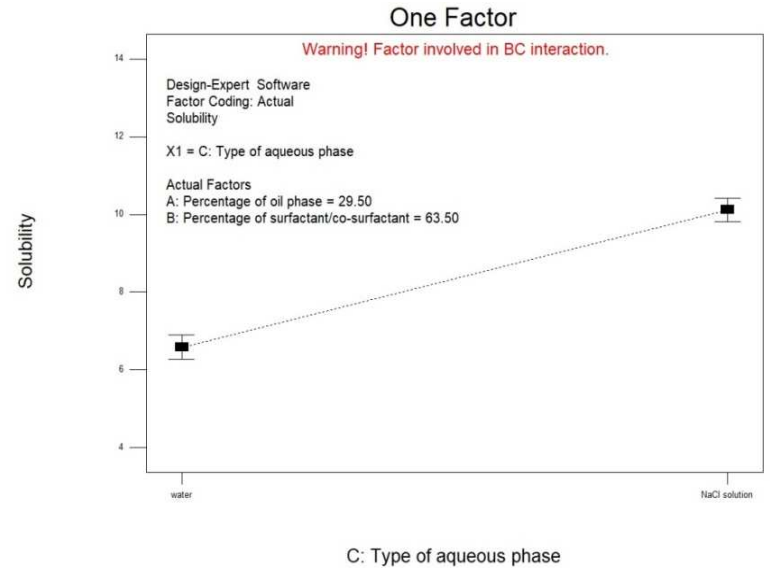
B: Percentage of surfactant/co-surfactant

B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X1 = C: Type of aqueous phase
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water
NaCl solution
C: Type of aqueous phase

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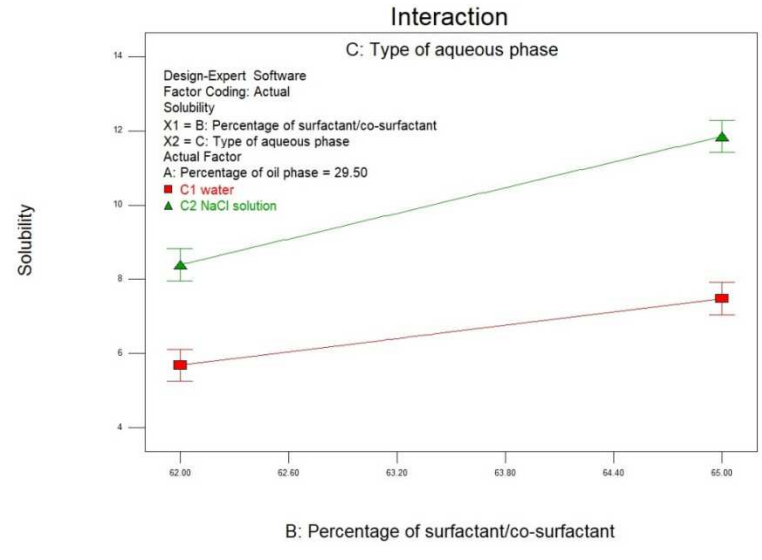

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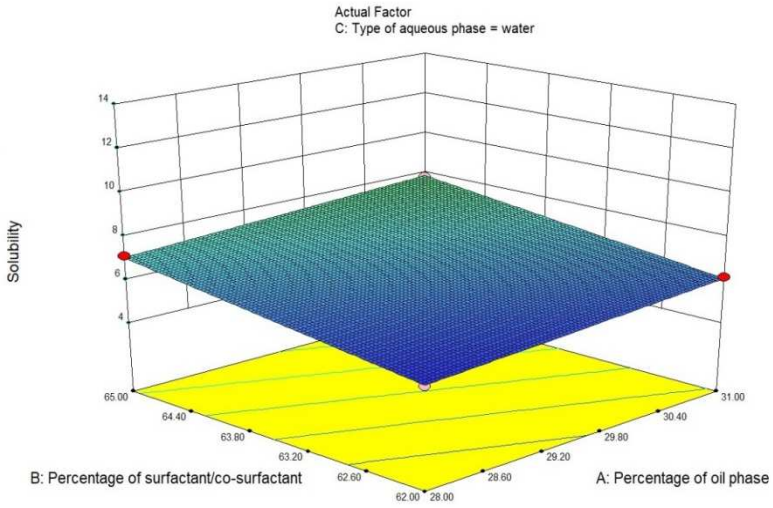

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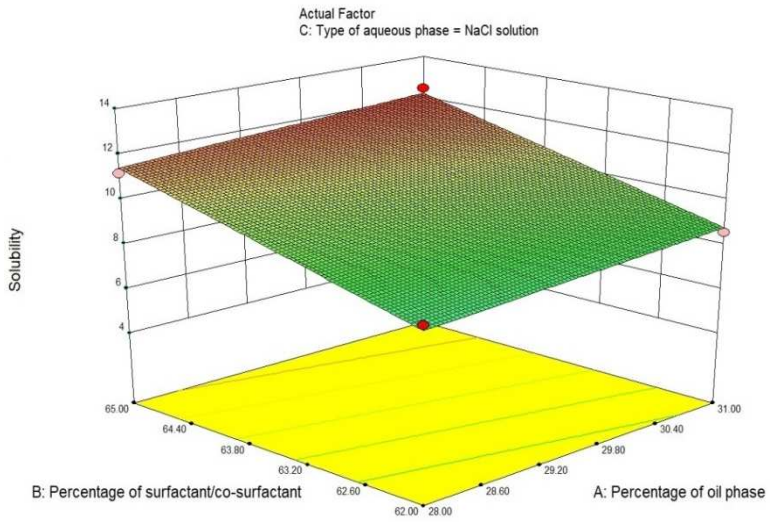
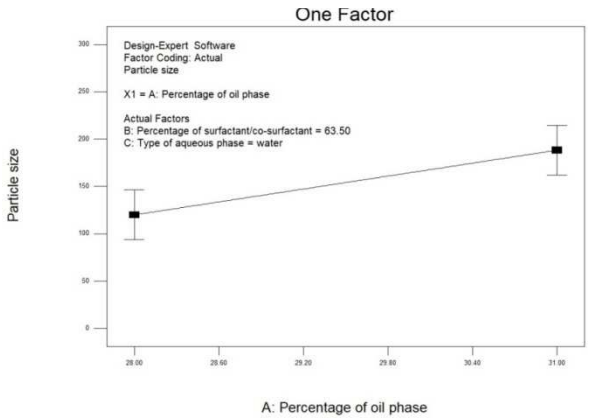


F

Fig. 3: Factorial analysis of the effect of individual variables and their interactions on quercetin solubility of microemulsions (A) effect of variable A (percentage of oil phase) (B) effect of variable B (percentage of surfactant/co-surfactant) (C) effect of variable C (type of aqueous phase) (D) effect of B×C interaction (E) effect of variable A and variable B on solubility (water as an aqueous phase) (F) effect of variable A and variable B on solubility (NaCl solution as an aqueous phase)



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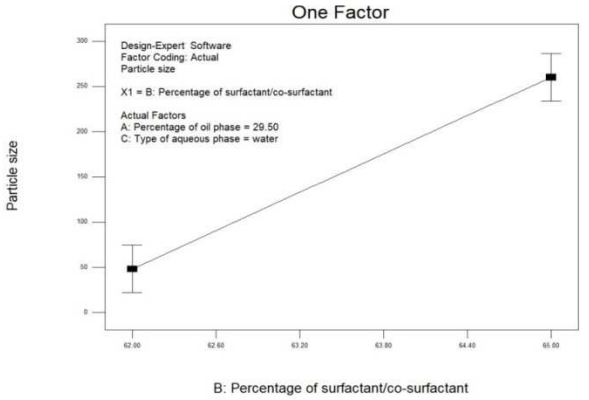

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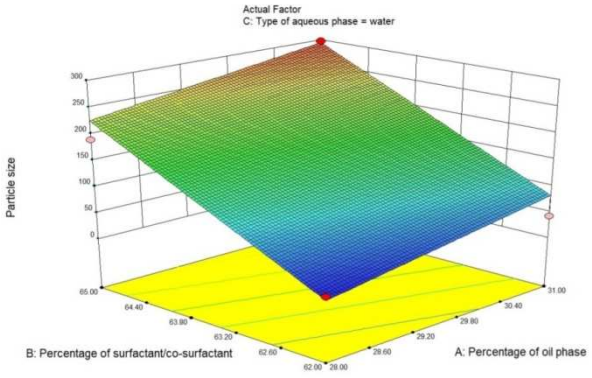

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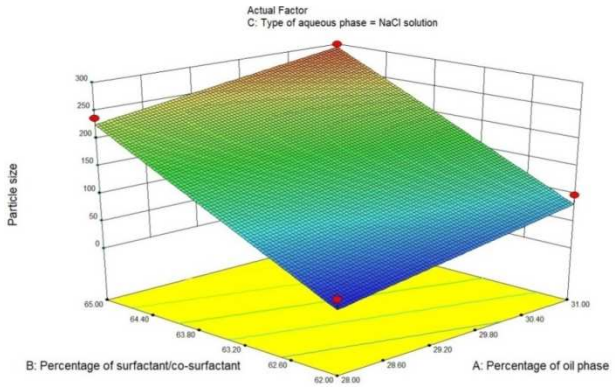


D

Fig. 4: Factorial analysis of the effect of individual variables on particle size of microemulsions (A) effect of variable A (percentage of oil phase) (B) effect of variable B (percentage of surfactant/co-surfactant) (C) effect of variable A and variable B on particle size (water as an aqueous phase) (D) effect of variable A and variable B on particle size (NaCl solution as an aqueous phase)

In addition to identifying individual variables, the interactions are the key to getting the optimal conditions. The results obtained from the interactions are shown in fig. 3D and can be described as follows. From the BC interaction, regardless of whether the variable C (type of aqueous phase) was at low level or high level, that is to say despite the difference in aqueous phase, increasing the variable B (percentage of Smix) will cause the increasing of quercetin solubility. Furthermore, the percentage of Smix (variable B) has little effect when the aqueous phase (variable C) is at the low level in contrast to the high level. Increasing variable B at a low level of variable C tended to increase the solubility of quercetin, but the influence was minor compared to that at a high level of variable C. Similarly, variable C has a more significant effect when variable B is at the high level in contrast to the low level.

On the other hand, the analysis results of particle size are shown in fig. 2 and fig. 4. The variable A and B are obviously the effective variables while variable C has no significant effect on particle size. There is no obvious interaction between the three variables. In addition, it can be seen in fig. 4 A-D that the two variables have a positive effect on the particle size of microemulsions. Increasing the oil phase and the percentage of Smix led to an increase in the particle size of microemulsions from 120.08 nm to 188.38 nm and 48.18 nm to 260.28 nm, respectively. The influence of variable B (percentage of Smix) is quite significant (fig. 4B). Because the surfactant was adsorbed on the interface of the microemulsion droplets, the long lipophilic chain of the surfactant might increase the droplet size of the microemulsion [17]. Therefore, for variable B (the percentage of Smix), the particle size of the microemulsions will increase relatively at a larger Smix ratio. Besides, the solubility of quercetin increases by a larger percentage of Smix. It can be assumed that more quercetin accumulates on the interface layer of the droplet and stays in the continuous phase, and larger particles will be produced at higher quercetin concentrations [36-38]. This phenomenon can also explain the influence of variable A on particle size: increasing the percentage of the oil phase will also increase the solubility of the hydrophobic quercetin, resulting in an increase in particle size.

***In vitro* release studies**

The thermodynamic activity of the drug in the formulation is an important driving force for drug release. A high-concentration formulation should have a higher thermodynamic activity [39, 40]. Therefore, based on the results obtained from the previous experimental analysis, the formulations for the maximum solubility of quercetin prepared with water and NaCl solution respectively (ME-4 and ME-8) were selected and *in vitro* release studies were performed to compare the release of quercetin from the two microemulsions. The results obtained are shown in fig. 5. Both microemulsions showed good *in vitro* drug release. The release data were analyzed as per zero-order, first-order and Higuchi models. The zero-order kinetic model describes the system where the drug release rate is only a function of time and independent of its concentration. In contrast, the first-order kinetic model is indicative of systems where drug release is a concentration-dependent process. Higuchi model describes the release of drugs from the formulation is proportional to the square root of time. The Higuchi model had the best fit with a higher correlation for two formulations (fig. 6A and 6B). The release of quercetin was linear when the amount released percentage was plotted as a function of the square root of time. The correlation coefficients (R^2) of Higuchi model were 0.9796 and 0.9809, respectively. For other kinetic models, the correlation coefficients of the zero-order model were 0.8437 and 0.8573, as well as the first-order model were 0.6645 and 0.6957.

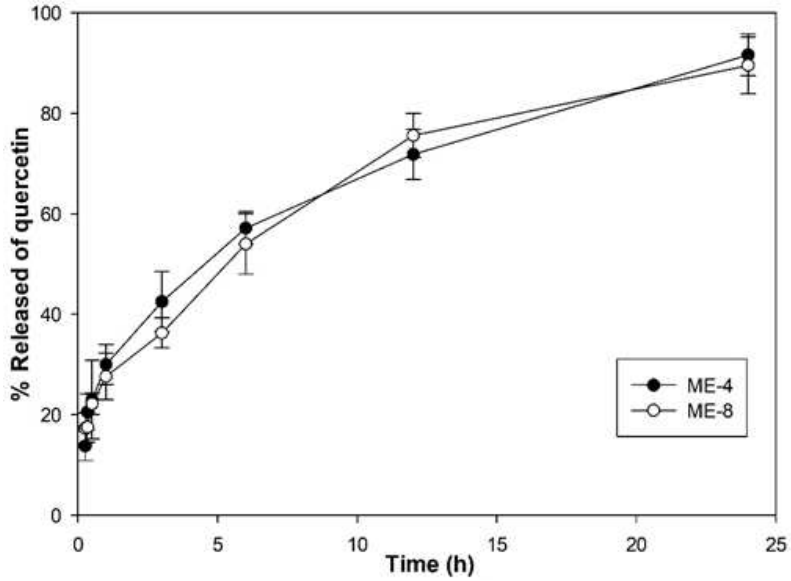


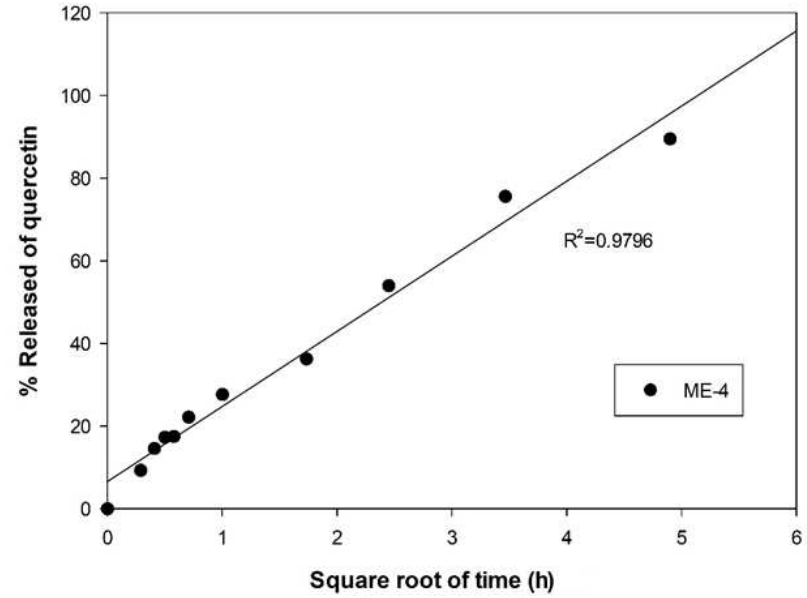
Fig. 5: *In vitro* release profiles of quercetin from two microemulsions (ME-4, ME-8). Values are expressed as mean±SD (n=3)

Stability test

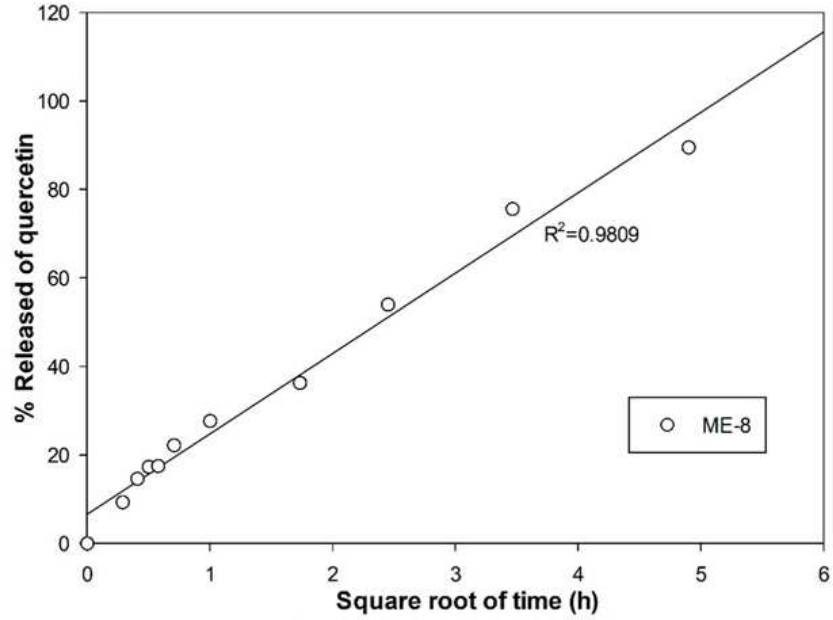
The stability assessment of the active ingredients in the formulation is important for the development of new products. Storage at different climatic conditions may affect the effectiveness of the product [31, 40]. Chemical stability (change in drug content) studies can evaluate the ability of a drug to ensure its efficacy and safety at the necessary concentration. However, considering the fact that antioxidant formulations could become pro-oxidant or lose its activity without apparently altering the drug content, the functional stability assessment should be accompanied by chemical stability studies to understand whether the anti-oxidation and anti-tyrosinase activities of quercetin are affected in different storage environments [41]. Functional stability guarantees the efficacy of products with specific functions. In this study, in addition to evaluating the changes in quercetin content of microemulsions stored at three different temperatures, two functional stability (DPPH scavenging, anti-tyrosinase activity) are also used as another method to evaluate the stability of quercetin in microemulsions.

Fig. 7 shows the change of quercetin content in microemulsions in different storage environments, and the quercetin solution is used as a reference for comparison. Regarding the stability of the microemulsions at different temperatures, the quercetin microemulsion stored at 4 °C showed chemical stability during the entire experiment (3 mo). The quercetin concentration of microemulsion stored at 30 °C decreased slightly, while the quercetin concentration of microemulsion stored at 40 °C decreased

significantly. However, the quercetin content in the quercetin solution stored at different temperatures decreased significantly in the first month, and the quercetin content in the third month decreased by more than 40%. It can be observed that the microemulsion increases the stability of the content over a longer period of time, and the quercetin in the microemulsion shows better stability than the quercetin solution in three different storage environments. Temperature conditions may change the hydrophilic-lipophilic balance of surfactants and destabilize the surfactant interface, affecting the formation of microemulsions and the stability of quercetin. This phenomenon can be confirmed by the functional stability results, that quercetin microemulsions have better functional stability than quercetin solution (fig. 8 and 9). The microemulsion maintained its antioxidant and anti-tyrosinase activity when stored at 4 °C during the 3 mo of analysis. The microemulsions under other conditions, especially 40 °C, had a significant decrease of antioxidant and anti-tyrosinase activity. Stability of functional activity at 4 °C was better than other storage temperatures, which might be due to the fact that quercetin was most stable at 4 °C. When comparing the concentration of quercetin and antioxidant activity, the reduction in antioxidant activity was not as obvious as the reduction in quercetin concentration. These differences can be explained by the fact that some of the quercetin degradation products may have antioxidant activity. These results indicate that care must be taken with the storage conditions of these formulations to maintain its safety and functionality [37].



A



B

Fig. 6: Fitting experimental data of microemulsions by higuchi release kinetic model (A) ME-4 (B) ME-8, values are expressed as mean±SD (n=3)

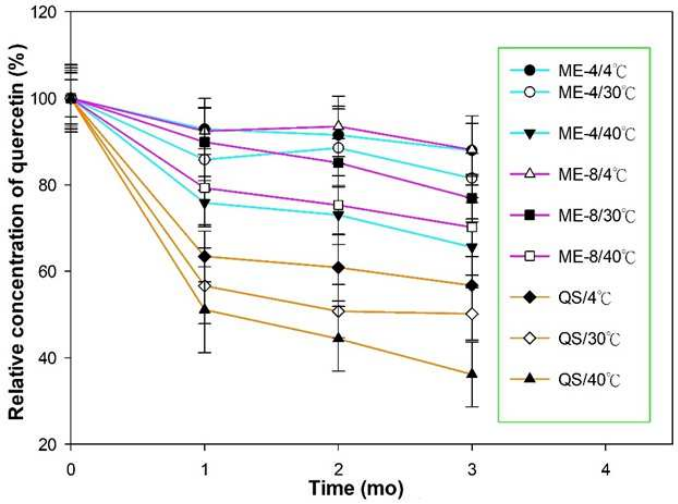


Fig. 7: Chemical stability of quercetin in microemulsions (ME-4, ME-8) and aqueous solution (QS) stored at 4 °C, 30 °C, and 40 °C for 3 mo. Values are expressed as mean±SD (n=3)

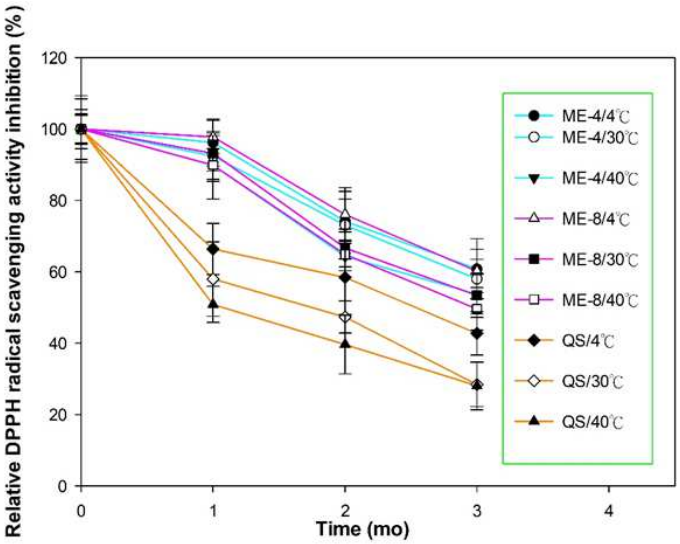


Fig. 8: Antioxidative stability of quercetin in microemulsions (ME-4, ME-8) and aqueous solution (QS) stored at 4 °C, 30 °C, and 40 °C for 3 mo, values are expressed as mean±SD (n=3)

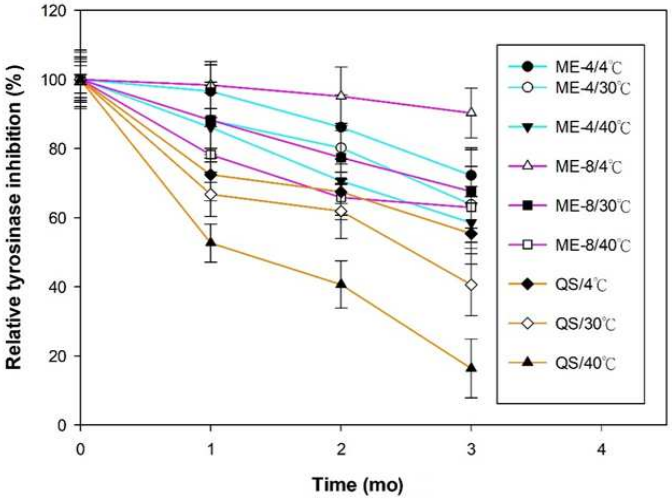


Fig. 9: Anti-tyrosinase stability of quercetin in microemulsions (ME-4, ME-8) and aqueous solution (QS) stored at 4 °C, 30 °C, and 40 °C for 3 mo, values are expressed as mean±SD (n=3)

CONCLUSION

In this study, the influence of each variable on the characteristics of microemulsions was complicated. Increasing the percentage of oil phase and the percentage of Smix could increase the solubility of quercetin in microemulsions, but it also caused an increase in particle size. The type of aqueous phase has a significant effect on the solubility of quercetin in microemulsions, but has no apparent effect on the particle size of microemulsions. Increasing the solubility of quercetin in microemulsions is beneficial to increase the drug that can penetrate into the skin, but increasing the particle size results in poorer skin penetration. The result demonstrates that such details can ascertain critical parameters that have to be controlled in order to give optimal formulation. In addition, microemulsions could protect the embedded drugs from degradation and maintain fine antioxidative and anti-tyrosinase activities at an appropriate temperature, which was very useful for the development of delivery systems.

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Nil

AUTHORS CONTRIBUTIONS

All authors contributed to the study conception and design. YK Lo and YT Huang performed the experiment and analysis. WH Chuo and CS Wu performed the interpretation. The first draft of the manuscript was written by WH Chuo and the critical revision of the manuscript was done by CS Wu. All authors read and approved the final manuscript.

CONFLICT OF INTERESTS

This statement is to declare that all authors involved in this manuscript have no conflict of interest.

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