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RESEARCH ARTICLE

Exploration of probiotics on antioxidant activity of serum NLC coenzyme Q10 using the DPPH (2,2-diphenyl-1-picrylhidrazyl) method

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Abstract

Background: The skin ageing process can be slowed by routinely caring for the skin using serum cosmetics containing antioxidants such as Co-enzyme Q10 (CoQ10). However, CoQ10 has a drawback, which is low penetration into the skin. In this study, an antiaging serum preparation with the active ingredient NLC-CoQ10 was prepared. Probiotics were added to this preparation as a second active ingredient to enhance antioxidant activity. **Objective:** This study aimed to analyse the effect of probiotic concentration on the physical characteristics and antioxidant activity of NLC-CoQ10 serum. **Method:** NLC-CoQ10 was mixed with serum base and 0% probiotic (Formula One), 2% probiotic (Formula Two), and 4% probiotic (Formula Three). After that, physical characteristics and antioxidant activity were observed. **Results:** The results show that the physical characteristics of all formulas fulfil the specifications. All formulas had pH values between 4 and 7 and a viscosity value between 230 and 1150 cPs. The formula with the highest viscosity value is F1, 352.00 ± 2.00. All formulas have statistically different IC_{50} values. IC50 values for F1, F2 and F3 respectively, are 317.809 ± 2.117; 307.985 ± 2.089; 257.549 ± 1.573. **Conclusion:** The antioxidant activity increases with increasing concentrations of probiotics in NLC-CoQ10 serum. Meanwhile, the viscosity value decreases with a decreasing concentration of probiotics.

Introduction

The ageing process occurs based on age, but some age more quickly, commonly referred to as premature ageing (Wardaniati & Islami, 2020). This can be caused by the excess production of ROS and melanin in the skin, which induces skin ageing (Matos *et al.*, 2019). One of the strategies to fight skin ageing is to use antiaging cosmetics such as serums (Wink, 2022).

Serum with a good formula can firm the skin and make it smoother. It can also diminish pores and increase moisture levels. Serum contains several beneficial active components such as antioxidants, ceramides, and amino acids (Sasidharan *et al*., 2014). All skin types need antioxidants to make skin as healthy as possible, such as Coenzyme Q10 (CoQ10) (Raizner, 2019).

CoQ10 is a fat-soluble material that can prevent lipid per-oxidation, collagen and elastin damage, and wrinkles on the skin; Korkmaz *et al.*, 2013). CoQ10 potentially prevents skin damage due to photo-aging (Yue *et al.*, 2010). However, CoQ10 has several drawbacks, including low penetration to the skin and easily degraded by light and high temperatures (Bao *et al.*, 2019). To overcome these deficiencies, it is necessary to formulate CoQ10 into nanostructured lipid carriers (NLC).

NLC is a nano-sized drug delivery system generally composed of solid lipids, liquid lipids, and emulsifying agents (Kaur *et al.,* 2015). Some of the advantages of NLC include the high entrapment power of the active ingredients and the low expulsion of the active ingredients from the lipid matrix during storage. NLC can protect the active ingredients in the lipid matrix so

the stability of the active ingredients is increased (Müller *et al.,* 2014).

In this study, an antiaging serum preparation with the active ingredient NLC-CoQ10 was prepared. Probiotics were added to this preparation as a second active ingredient. In recent years, probiotics used as active ingredients in the skincare (Pratiwi & Susanti, 2021; Pratiwi & Susanti, 2023). The cosmetic industry which develops skincare products has recognised the importance of probiotics as "*bioactive ingredients*" in enhancing the beauty. Moreover, other benefits of probiotic cosmetics are able to accelerate the process of skin rejuvenation, and protect against ultraviolet (UV) rays (Lew & Liong, 2013). The purpose of this study was to analyse the effect of probiotic concentration on the physical characteristics and antioxidant activity of NLC-CoQ10 serum.

Methods

Materials

The materials used in this study included Probiotics (Lactobacillus bulgaricus), CoQ10 (Kangcare), beeswax (Xiamen), Illipe butter (Gunung Hijau Masarang), jojoba oil (Jojoba Desert), Tween 80 (Brataco Chemical), Span 80 (Kolb), Propylene glycol, aquademineral (Brataco Chemical), butylene glycol, ethanol pro analysis/ethnol p.a. (Smartlab Indonesia), 2.2 Diphenyl-1-Pikrihydrazil/DPPH (Sigma Aldrich). Table I showed the NLC-CoQ10 serum formula with probiotics.

Table I: NLC-CoQ10 serum formula with probiotics

Components	Formula 1 (%)	Formula 2 (%)	Formula 3 (%)
NLC-CoQ10*	50	50	50
Probiotics	0	2	4
Serum base†	ad 100	ad 100	ad 100

*NLC-CoQ10 consisted of: CoQ10 (2%), beeswax (0.990%), illipe butter (2.970%), jojoba oil (2.640%), Tween 80 (13.604%), Span 80 (6.896%), Propylene glycol (3.5%), BHT (0.1%), phosphat buffer pH 6.0.
†The serum base consisted of xanthan gum (0.4%), Butylene glycol (10%), aquademineral (ad 100).

Physical characteristic testing organoleptic

The organoleptic test was carried out visually. The observations recorded included the consistency, color, and smell of the sample test.

pH

The test was carried out using a digital pH meter (Eutech instrument pH-meter pH 2700). The sample test was diluted using distilled water with a ratio of 1:9. Then, the electrode was dipped in the sample solution. (Mayangsari *et al.,* 2022).

Viscosity

The test was carried out using a Brookfield Viscometer (Brookfield DV1 - RV). The sample (300g– 500g) was put into a beaker glass. Then, the spindle was installed. After that, the highest rotational speed was selected. If the torque value was more than 100%, then the test was repeated with a lower rotational speed and/or smaller spindle, vice versa, if the torque value was less than 10% (Mayangsari *et al.,* 2022).

Particle size, polydispersity index (PI), and Zeta potential

Particle size, PI, and Zeta potential were carried out using Zetasizer nano (Malvern Instrument). The sample (50mg) was put into a beaker glass. Then, added aquademineral to a volume of 50.0mL. It was stirred using a magnetic stirrer at 500rpm for 10 minutes. Then, the solution was taken as much as 2.0ml and then added 8mL of aqua demineral. It was stirred again using a magnetic stirrer at 100rpm for ten minutes. The test sample (which was diluted) was put into the cuvette, then the particle size index, PI, and zeta potential were observed (Fachriani *et al.,* 2022).

Antioxidant activity

The antioxidant activity was carried out using the DPPH method. The steps were adapted from a previous study conducted by (Wulansari *et al*., 2022) with several modifications. A total 2ml each solutions series concentration of F1, F2 and F3 was put into test tube. Then, 2mL of DPPH solution (50ppm) was added, homogenised, and incubated in a dark room for 30 minutes. The absorbance was measured using UV-Vis spectrophotometer at the maximum wavelength. % inhibition was calculated using equation (1). The IC50 value was calculated from linear regression between % inhibition and concentration.

Equation (1): $\%\ Inhibition = \frac{A-B}{A} \times 100\%$

A= absorbance of DPPH
B = absorbance of sample

Data analysis

The data was analysed using descriptively and one way ANOVA.

Results

Physical characteristics of NLC-CoQ10 and Serum base physical characteristic of NLC-CoQ10 are presented on Table II. While, physical characteristic of serum base are presented on Table III. Based on the tables, it can be conclude that all parameters fulfill the specification.

Table II: Physical characteristics of NLC-CoQ10

Parameters	Specification	Results
Organoleptic	Light orange, odorless, liquid form	Light orange, odorless, liquid form
pH	4–7.0	5.87 ± 0.02
Particle size	< 1000	199.8 ± 2.21 nm
Polydispersity index	< 0.5	0.37 ± 0.01

Table III: Physical characteristic of serum base

Parameters	Spesification	Results
Organoleptic	Clear, odorless, a little thick	Clear, odorless, a little thick
pH	4–7.0	5.85 ± 0.04
Viscosity	230-1150 cPs	744.33 ± 3.05 cPs

Physical characteristics of F1, F2 and F3

Organoleptic of F1, F2 and F3 are presented on Table IV. The color of the test preparation can be seen in the Figure 1. Based on Table IV, it can be concluded that all test preparation had the same color, odor, and consistency. pH value of F1, F2 and F3 are presented on Figure 2. Based on statistical analysis using one way anova, it is known that the pH values of the three preparations were not significantly different, as evidenced by a significance value of 0.407 (>0.05). Viscosity value of F1, F2 and F3 are presented on Figure 2. Based on statistic analysis using one way anova, a significance value of 0.000 was obtained. Then, the test was continued with post hock tukey HSD. From these tests, it is known that all formulas were significantly different, as evidenced by a significance <0.05. Even though the viscosity values of the three preparations were different, they had the same consistency (Table IV).

Table IV: Organoleptic of F1, F2, F3

Observations	F1	F2	F3
Color	Light yellow	Light yellow	Light yellow
Odor	Odorless	Odorless	Odorless
Consistency	A little thick	A little thick	A little thick

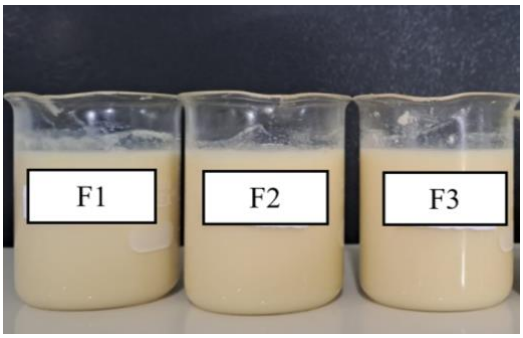

Figure 1: CoQ10 NLC preparation with probiotics

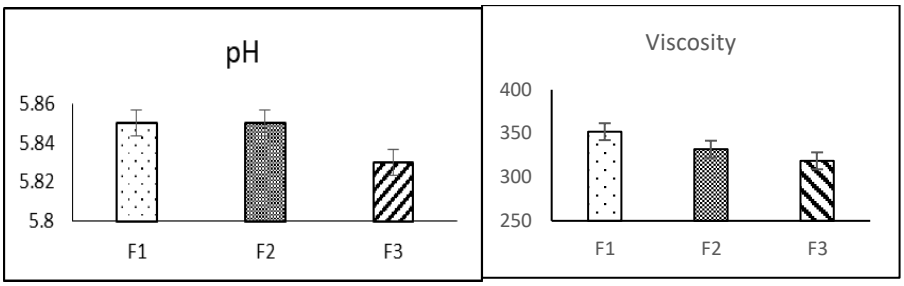

Figure 2: pH and viscosity test results of CoQ10 NLC with probiotics (n=3). *p < 0.05 all formulas were significantly different.

Antioxidant activity of F1, F2 and F3

The IC_{50} values of F1, F2 and F3 F3 are presented on Table V. The value were statistically analysed using one way anova to obtain the differences in the IC_{50} values in the three formulas. Based on the test, it is known that the significance value was 0.000. Testing continued using the post hock tukey. As a result, it is known that the three formulas occupied different subset columns. This means that the IC_{50} values of F1, F2 and F3 were significantly different.

Table V: IC_{50} values for F1, F2, F3

Formula	IC_{50}
F1	317.809 ± 2.117*
F2	307.985 ± 2.089*
F3	257.549 ± 1.573*

Note: *p < 0.05 all formulas had significant differences

Discussion

Based on the particle size test, it is known that the particle size of NLC-CoQ10 met the desired specifications. NLC particle size requirements were below 1000 nm (Khosa *et al.,* 2018). Based on PI value, it is known that NLC-CoQ10 met the desired specifications. Formulation of nanoparticles with a PI value of less than 0.5 was considered to have a homogeneous and monodisperse particle size. Whereas those with polydispersity index values greater than 0.5 were considered to have inhomogeneous particle sizes and polydispersity (Supriya *et al.,* 2021). The pH value of NLC-CoQ10 and serum base met the normal pH specifications of the skin, namely 4.5 – 7.0 (Fachriani *et al.,* 2022).

The pH value of F1, F2, F3 met the normal pH specifications of the skin, namely 4.5 – 7.0 (Fachriani *et al.,* 2022). The viscosities of F1, F2, F3 were in the range 230-1150 cPs. Based on (Mardhiani *et al.,* 2018), the viscosity of ideal serum between 230-1150 cPs.

All antioxidant activity tests observed at a maximum wavelength of 515 nm. DPPH solution gave the maximum absorbance value (0.6353) at a wavelength (λ) of 515 nm so that the maximum λ of DPPH was 515 nm. Nanaei and authors mentioned that the maximum λ of DPPH was about 515 nm - 520 nm (Nanaei *et al.*, 2019).The concentration of the test solution included 12.5; 25; 50; 100; 200 ppm. Percent inhibition obtained from each concentration was plotted in the linear regression equation (y = a ± bx). IC_{50} calculation results can be perceived in Table V.

The IC_{50} values of F1, F2 and F3 were statistically analysed using one way anova to obtain the differences in the IC_{50} values in the three formulas. Based on statistical analysis using one way ANOVA, it is known that the significance value was 0.000. Testing continued using the post hock tukey. As a result, it is known that the three formulas occupied different subset columns. This means that the IC_{50} values of F1, F2 and F3 were significantly different. The lowest IC_{50} value was the IC_{50} value from F3 (257.549 ± 1.573), while the highest IC_{50} value was the IC_{50} from F1 (317.809 ± 2.117). This proves that increasing the concentration of probiotics affected the IC_{50} value. The higher the concentration of probiotics added, the lower the IC_{50} value obtained. The antioxidant activity increases with increasing concentration of probiotics in NLC-CoQ10 serum.

Based on Table V, it can be concluded that F1, F2, and F3 had weak antioxidant activity. Although serum containing NLC CoQ10 and probiotics had weak antioxidant activity in vitro, but in vivo it was not necessarily bad. Pratiwi and author conducted research related to testing the effectiveness of creams containing probiotics on repairing skin exposed to UVC rays for 28 days. As a result, it was perceived that there was a difference between the wrinkles on the backs of mice that were given placebo and the backs of mice

that were given probiotic cream (Pratiwi & Susanti, 2023).

Conclusion

The antioxidant activity increases with increasing concentration of probiotics in NLC-CoQ10 serum. While viscosity value decreases with decrasing concentration of probiotic. This research is expected to give contribution in the development of cosmetic science.

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