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Submission date: 11-Jun-2019 12:22PM (UTC+0800)

Submission ID: 1142415333

File name: C-12.pdf (230.14K)

Word count: 2608

Character count: 13721

Photostability Study on Character and Antioxidant Activity of Tomato Extract (*Solanum lycopersicum L.*) in Nanostructured Lipid Carrier (NLC) and Conventional Creame

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Received: 18th Dec, 2016; Revised: 10th Feb, 2017; Accepted: 25th Feb, 2017; Available Online: 1st March, 2017

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ABSTRACT

The aim of this study was to investigate the ability of NLC in increasing photostability of tomato extract in term of antioxidant activity. Photostability testing on antioxidant activity of samples were conducted by accelerating method using UV_B radiation 32.400 joule for 21 hours radiation. Antioxidant activity was measured by DPPH method. NLC was made by High Shear Homogenization (HPH) method at 24000 rpm for 4 cycles, while conventional creame was made by low speed at 400 rpm. The product were characterized include: pH, viscosity, and particle size. There were had difference characters and physical stability. NLC had smaller size, more homogenous and more stable than conventional creame. It was known that stability of antioxidant activity of tomato extract in NLC system higher than in conventional creame. That was showed with k value, as constanta of rate scavenging activity decreasing in antioxidant power between time (Sigma 2-tail < 0.005) of NLC and conventional creame were: $2.03 \times 10^{-2} \% / \text{hour} \pm 0.08$ (3.94) and $4.71 \times 10^{-2} \% / \text{hour} \pm 0.23$ (4.88) respectively.

Keywords: antioxidant, photostability, tomato extract, NLC, DPPH.

INTRODUCTION

The aging process of the skin consists of two processes, namely intrinsic aging and extrinsic aging. Intrinsic aging is the aging process that occurs naturally, caused by genetic factors, hormonal and racial. Extrinsic aging process is a process that is caused by factors outside the body such as sunlight (UV rays)¹. Photobiology effect of UV rays (UV_A and UV_B) produce free radicals that cause damage to the skin². It is therefore that the body needs antioxidants to protect the body from free radical attack³. The antioxidant protect the enzymes that repair DNA damages, thereby enhancing our body's ability to rejuvenate itself⁴. One antioxidant is derived from tomato extract (*Solanum lycopersicum L.*). Tomato extract is a natural source of antioxidants, especially lycopene as the highest content. Lycopene is one of about 600 types of carotenoids that act as antioxidants. This compound is a natural color forming pigment contained in tomatoes. Tomato extracts is unstable against light, heat, degradan and poorly soluble in water. As topical cosmetic preparations, a proper delivery system to improve the stability of the tomato extract was needed. One of delivery system was selected is *Solid Lipid Nanoparticle* (SLN). *Solid Lipid Nanoparticle* (SLN) is colloidal dispersion in nano size consists of a solid lipid stabilized by surfactants that work in the interface area⁵. Some advantages of SLN, for instance the ability to protect the active compounds from degradation, controlled release, the ability to load a

lipophilic or hydrophilic material, non-toxic and did not irritate.⁵ Limitations of SLN systems included loading capacity of the active ingredient was limited and the expulsion of active ingredient during storage. This was because of the high orderdness of the lattice crystal of solid lipid so that the active ingredient which has been entrapped easily squeezed out of the system. To overcome the limitations of the system SLN, a second-generation nanoparticle system that is *nanostructured lipid carrier* (NLC) was developed^{6,7}. NLC are formed with both liquid and solid lipid as a blend mixture in such a ratio that they are solid at room temperature. The active ingridient can be incorporated in particle matrix in a molecular dispersed form or it can be arranged as amorphous cluster⁶. SLN and NLC system were both ideal carrier cosmetic preparations because they contained lipid base provide emollient effect on the skin. The existence of liquid lipid decreased in the ordermess of solid lipid crystal lattice so that the active ingredient entrapped more stable and increased drug loading^{8,9}. The active ingredients that are entrapped in the system was expected to increase its stability and less contact with the free radicals exposed in the air. This research examined the influence of the NLC system against antioxidant stability of tomato extract which was compared to conventional creame. The parameters used to determine the stability of the antioxidant extract of tomato in the system, based on the value of a constant percent scavenging activity decreasing in antioxidant power

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between time (*k*). Stability test was done by acceleration test by 32,400 Joule UV_B rays exposure for 2, 5, 9, 15 and 21 hours.

MATERIALS AND METHODS

Materials

Tomato extract (the Asian Group CN Nutrition Lab), alcohol, distilled water and methanol, olive oil (PT Safarindo Internusa Jakarta), Kolliphor® P188 (Poloxamer 188) and tween 80 both were obtained from Sigma Aldrich.

Methods

Measurement of Tomato Extract Antioxidant activity (IC₅₀) by DPPH method^{4,10}. Each tomato extract solutions with various concentration⁵ was added with 0.004% of DPPH solution and then allowed to stand for 30 minutes after the measurement of absorbance at a wavelength of maximum DPPH at three wavelengths, namely 505, 515, and 525 nm using the formula: Absorbance Calculate at 515 nm = $A_{515} - \frac{A_{505} + A_{525}}{2}$(1)

Note: A₅₁₅ nm = Absorbance of the sample solution at a wavelength 515 nm

A₅₀₅ nm = Absorbance of the sample solution at a wavelength 505 nm

A₅₂₅ nm = Absorbance of the sample solution at a wavelength 525 nm

A control = Absorbance of the DPPH solution as blanko

A sample = Absorbance of the sample solution

% free radical inhibition = $1 - \left(\frac{\text{Absorbance Calculate of sample}}{\text{Absorbance Calculate of DPPH}} \right) \times 100\%$(2)

To determine IC₅₀ curves made between % inhibition data obtained as ordinate with concentration as absis. IC₅₀ value obtained by entering y = 50 into the regression equation y = bx + a wich was obtained from the curve of the relationship between free radical inhibition data to concentration, in order to get the value of x as the value IC₅₀.

NLC and conventional creame Preparation

NLC was made by diluting the tomato extract to molten lipid phase consisting of solid lipid and liquid lipid at 60 °C ± 5 °C (speed of 3,400 rpm for 2 minutes). The aqueous phase was added to the mixture was stirred for 5 minutes. Further stirring at a speed of 24,000 rpm for 8 minutes used Ultra Turax IKA® T-25 Digital High Shear Homogenizer. The next step was cooling process until it reached room temperature. Preparation of conventional creames did like the NLC preparation but made with a hot plate Dragon Lab MS H-Pro with low speed (400 rpm). The composition of formula NLC and conventional creames can be seen in table 1.

Sample Characterizations were done by measuring pH, viscosity, particle size, Polydispersity Index (PI)

Determination of pH

Determining pH of each test preparation was done using a pH meter.

Viscosity Measurement

Viscosity measurement was done using a Brookfield Cone and Plate Viscometry®. It was performed for each sample by diluting the sample 1: 9 with CO₂ free aquadest.

Table 1: Formula NLC and conventional creame (CC).

Ingredient	Function	Concentration (%w/w)	
		NLC	Conventional Creame (CC)
tomato extract (TE)	active ingredient	0.25	0.25
cetyl alcohol	solid lipid	18	18
olive oil	liquid lipid	2	2
Poloxamer 188	surfactant	5	5
Tween 80	surfactant	5	5
aquadest	water phase	ad 100	ad 100

Table 2: Characterisation of NLC and conventional creame.

Sample	Characterisation of NLC and conventional creame ± SD (CV)	
	pH	Viscosity
NLC	5.59 ± 0.02 (0.27)	2,416.33 ± 186.50 (7.72)
conventional creame	5.29 ± 0.03 (0.47)	1,563.33 ± 85.82 (5.50)

Table 3: The constanta of rate scavenging activity decreasing in antioxidant power.

Sample	Mean constanta of rate scavenging activity decreasing in antioxidant power (%/hour) ± SD (CV)
NLC	2.03x10 ⁻² ± 0.08 (3.94)
conventional creame (CC)	4.71x 10 ⁻² ± 0.23 (4.88)
Tomato extract (TE)	4.41x10 ⁻² ± 0.2 (4.53)

Examination of the particle size and size distribution

Examination of the particle size consisting of two treatments before and after UV_B irradiated for 21 hours. Each sample was diluted to 200 ppm stirring for 3 minutes at 500 rpm. Dilution checked by Delsa Nano Particle Size on the angle of 65° and a temperature of 25°C. Data obtained were average particle diameter and Polydispersity Index (PI). All measurement was replicated 3 times.

Antioxidant Activity Photostability Test

Antioxidant Stability for each dosage was calculated by the percent inhibition using formula 1. Tomato extracts in conventional creames and NLC were measured free radical activity inhibition percent after being exposed to UV_B rays produced from 32,400 Joule UV_B bulbs. The experimental conditions were controlled at 30 °C ± 5 °C. Free radical inhibition every 2, 5, 9, 15, and 21 hours after exposure to UV_B were determined. The results of calculations per cent inhibition between the time was made curve. Furthermore, *k* value as constanta of the rate of scavenging activity decreasing in antioxidant power were determined based on the appropriate reaction order.

RESULTS AND DISCUSSION

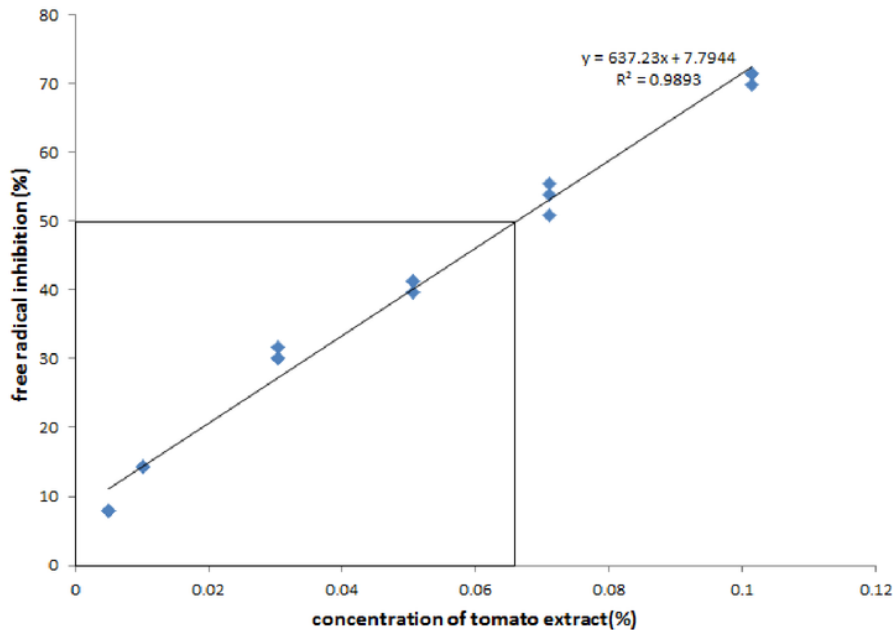


Figure 1: Curve of free radical inhibition of tomato extract.

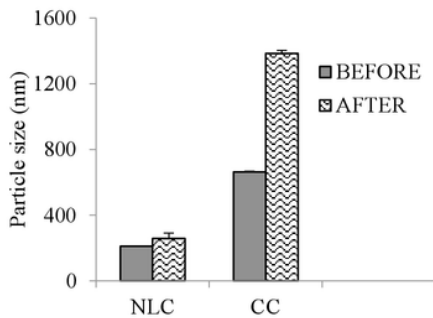


Figure 2: Histogram of average particle size of NLC and Conventional cream (CC) without and after exposure to UV_B for 21 hours.

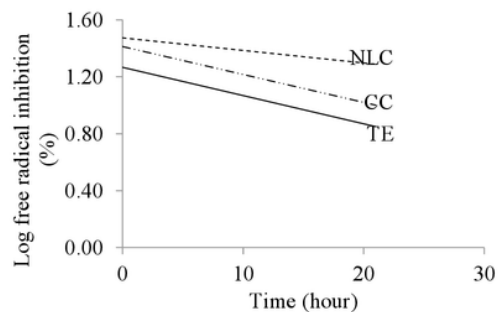


Figure 3: The curve of scavenging activity decreasing in antioxidant power.

Tomato Extract Antioxidant Activity

Antioxidants activity of tomato extracts were determined by DPPH method^{4,9}. DPPH free radical compounds are often used to test the antioxidant activity of several compounds or extracts of natural ingredients. DPPH compound is a free radical that has a purple color in solution. If there is a compound that can donate a proton, DPPH will be in a reduced form and its color intensity decreased. This study showed that the IC₅₀ of tomato extract was 0.0662% (Figure 1). This means that at 0.0662% tomato extract reduced 50% DPPH radical activity, or 0.14% tomato extract inhibited 100% free radicals. In this study, all samples used 0.25% tomato extract.

Physical Characteristic and Its Stability of NLC and Conventional Cream

The result of pH and viscosity of both samples were shown at Table 2. Both samples had an appropriate pH with skin (4.5 - 6.5). That was not irritant to the skin and comfortable to use. The viscosity of conventional cream was higher than NLC. It seemed associated with the particle size of the samples (Figure 2). The average particle size of NLC was 210 nm, while of conventional cream was about 662 nm approximately. It made NLC more viscous. The particle size of conventional cream is bigger than NLC because of the speed of mixing in conventional cream preparation was lower than NLC. Energy produced from mixing at low speed was not able to break the molten lipid droplets to be a small size. This was due to the energy produced was not capable to be distributed efficiently than when performed at high speed. By exposing UV_B radiation for 21 hours led to an increase in particle size. Greater increase occurred in the conventional

creames. The particle size of conventional creames was found to be 1,384 nm, while the NLC was 258 nm. It was caused by the energy of UV_B rays reduced the viscosity of the preparation, thus boosted the rate of flocculation of particles. The polydispersity Index (PI) NLC before and after irradiated <0.3. PI close to 0 indicated a homogenous dispersion, but when PI greater than 0.3 indicated a high heterogeneity¹⁰. It showed that the NLC had a homogeneous particle size. In conventional creames, UV_B exposure showed to degrade homogeneity of particle size, it showed with the change of PI value was 0.27 to be 0.42 *Antioxidant of Tomato Extract Photostability Test in NLC and Conventional Creame*

Antioxidant photostability test was conducted using accelerated method by exposure to UV_B radiation. The reduction of antioxidant activity over time can be seen in Figure 3. Stability antioxidants indicated by the value of *k*, as constant percent scavenging activity decreasing in antioxidant power between time. The greater the value of *k* indicated that the rate of scavenging activity decreasing in antioxidant power is high, it means that an antioxidant power was unstable. To determine the value of *k*, it had to known the order of the reaction previously. It was known that the rate of change percent reduction between the time progresses according to order 1. Order 1 means that the reaction rate was influenced one aspect, in this case was the content of active material¹¹. The following Table 3 contains the mean constant of rate scavenging activity decreasing in antioxidant power (%/hour) (*k*) samples. The value of *k* of tomato extract in conventional creame was not difference significantly with tomato extract itself, but greater than NLC. It showed that conventional creame could not be able to protect tomato extract from photo degradation. In NLC system, the active ingredient were entrapped in solid lipid, it would be protect the active ingredient from environmental influences.

CONCLUSIONS

It can be concluded that the NLC can improve the stability of the tomato extract antioxidant power than if only formulated in conventional creames.

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