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Encapsulation Process of Propolis Extract by Casein Micelle Improves Sunscreen Activity

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Abstract

Propolis, a wax bee glue, have antioxidant activity and effective as broad spectrum UVB and UVA photoprotection sunscreens agent. To improve photoprotection activity in sunscreen cream, the propolis was encapsulated by casein micelle. The Indonesian propolis was extracted by ethanol and separated the wax by freeze precipitation. The extract was encapsulated by casein and reduced their size by ball mill homogenizer with the encapsulation efficiency about 80% and size of particles about 80 nm. The particles was applied in sunscreen cream, evaluate their stabillity and photoprotection activity Sun Protection Factor (SPF) spectrophotometer (Optometric 290s). The results shows that the selected creams were stable and have moderate photoprotection activities higher than the controls. The encapsulation process of propolis by casein micelle can improve their photoprotection activity.

Abstrak

Proses Enkapsulasi Ekstrak Propolis dengan Misel Kasein dapat Meningkatkan Aktivitas Tabir Surya. Propolis adalah lilin yang berfungsi sebagai lem lebah, memiliki sifat antioksidan dan aktivitas tabir surya untuk daerah spektrum luas UVB dan UVA. Untuk meningkatkan aktivitas proteksi sinar UV di dalam krim tabir surya, propolis dienkapsulasi dengan misel kasein. Propolis Indonesia diekstraksi dengan menggunakan etanol dan dipisahkan lilinnya dengan presipitasi beku (*freeze precipitation*). Ekstrak propolis yang dihasilkan kemudian dienkapsulasi dengan kasein dan dikecilkan ukurannya dengan homogenizer *ball mill* dengan efisiensi enkapsulasi sekitar 80% dan dengan ukuran partikel sekitar 80 nm. Sediaan kemudian digunakan sebagai bahan aktif krim tabir surya, dievaluasi stabilitas krimnya dan aktivitas proteksi sinar UV-nya dengan menggunakan alat spektrofotometer sun protection factor (SPF) (Optometric 290s). Hasilnya menunjukkan krim terpilih stabil dan memiliki aktivitas proteksi sinar UV yang moderat dan lebih besar dibandingkan dengan krim kontrol. Proses enkapsulasi pada propolis dengan misel kasein terbukti dapat meningkat aktivitas proteksi sinar UV pada krim tabir surya.

Keywords: propolis, encapsulation, casein, photoprotection

1. Introduction

Propolis is a bee product of a complex resin that has vary of physical properties, depends on many factors [1]. The word propolis is taken from the Greek languange, “pro” means guards and the “polis” means a city. Generally, the propolis worksmantain bee colonies and its products from invading microorganisms [1].

Propolis is a natural product with antiseptics, anti mycotics, bacteriostatic, astringent, spasmolitik, anti inflammatory, anesthetic, antioxidant [2-4]. Propolis also has been used as an anti inflammatory and anti-bacterial drugs traditionally for centuries [5,6].

Propolis contains a various of compounds with bio-active components. The main compound of propolis are

flavonoids and polyphenols. There are also other compounds such as terpenes [7]. Recently, most of manufacturers are using ethanol as a solvent for propolis extract because it is more economical and easy to get. Currently, research is very much associated with propolis, starting from the content of propolis, propolis plant sources and it benefits. Propolis had antioxidant activity and it components can be used as sunscreen formulations (sunscreen). This development could be used as one of propolis products.

Another used of propolis as a supplement, whether liquid or solid. There's one issue about propolis and that is the nature of propolis properties can't be dissolve in water (hydrophobic) and it causes the body human being cannot abosorbed well because propolis properties has subtance like oil [8]. Because of this matters it need to be solve quickly in order to make propolis useful for bodies. To cause the bioactive compounds in the body can be optimally absorbed, there has been solutionin the processing propolis that can solve the problem. There is one technology that has existed is encapsulated with a coating casein micelle. These technologies apply the technique of encapsulation or coating using casein micelle. This encapsulation can be optimized change into nano-particles, it is called nano-encapsulation. Encapsu lation is a technique which of a substance or mixture of coated materials is been trapped in the system [9]. Coated material called active or core material and coating material called shell, wall material, carrier or encapsulant [9]. Thus this technique will be made nano-propolis encapsulated with casein micelle using high pressure ball mill homogenizer for making nano-sized particles.

The main purpose of production nanopropolis made using high pressure ball mill homogenizer is to obtain manufacturing methodsand produce potential beneficial that can be used as an active ingredient for the design of sunscreen cream products.

2. Materials and Methods

All the materials were analytical and cosmetical grades. The Casein and Propolis were extracted by Sahlan et al. method [10].

Encapsulation of propolis extract with casein. The encapsulation process was performed by Sahlan et al. method with slightly modified [10]. The 1.3 kg of casein wet weight was added a solution of phosphate buffer pH 10 in 5 liters, then stirred for 15 minutes, then add 1 liter of propolis, and then $CaCl_2$ 10% was added 100 ml every 5 minutes in six times, during the process of adding $CaCl_2$ a pH is maintained at pH 7 using 0.1 N HCl or 0.1 N KOH. Then the mixture solution was centrifuged so supernatant and sediment yield, the process is at the stage prior to encapsulation can be used as nano-sized particles called encapsulation with casein micelle (ePCM). The resulting precipitate propolis encapsulation will be made into nano-sized particles in crushed by using a High Pressure Ball Mill Homogenizer conducted in Nanotech Laboratory, Agency for the Assessment and Application of Technology (BPPT), Serpong. Then the precipitate which had become a nanopropolis with casein micelle (NePCM). NePCM re-dispersed in the resulting supernatant was 2.5 liters. Finally, the sample and the supernatant encapsulation nanopropolis analyzed encapsulation effi-ciency and particle size.

Encapsulation efficiency. The encapsulation efficiency performed to determine the ability of casein coating of the active compounds from propolis extract. Therefore, some tests that the test is active compounds flavonoids.

Method of aluminum chloride ($AlCl_3$) is used for determination of total flavonoids in propolis and the supernatant nanopropolis [11]. Quercetin was used as standards. EEP sample and the supernatant was pipetted nanopropolis of 0.5 mL and 1.5 mL of methanol was added, 0.1 mL of 10% $AlCl_3$ (m/v), 0.1 mL of 1 M of CH_3COOK and 2.8 mL of distilled water. After the incubation for 30 min at room temperature, then the absorbance of the sample can be measured using a spectrophotometer at a wavelength of 415 nm.

Nanoparticle size analysis. The size of nanoparticles were determined by Particle Size Analayzer (PSA), Delta™ Nano C, Beckman Coulter, conducted in Nano tech Laboratory, Agency for the Assessment and Application of Technology (BPPT), Serpong.

Sunscreen cream production. Carnauba wax or bees wax was mixed with olive oil, incubated on 60 ^oC until the wax melted and homogen with olive oil and mixed with hot water, added TiO_2 and decreased the temper ature until 40 ^oC and mix with hand mixer until homogen, added propolis and mix again.

Analysis the stabillity of cream. The stabillity of cream was monitored in physics and chemical properties. The physic stabillity was analyzed by organoleptic which monitored the stability of shape, color, odor, and homogenity. The chemical stability was characterize the stability of pH.

Photoprotection activity. The photoprotection activity were analysed by SPF spectrophotometer Optometric 290s. About 2 µl/cm2 of cream was spread in the trans-pore. The sample was radiated by ultra violet from 290 to 400 nm. Every samples radiated 12 times. The SPF number was calculated by Villalobos-Hernandez and Muller-Goymann method [12]. conducted in Research and Development Division, S&J International Enter-prises PC, Bangkok, Thailand.

3. Results and Discussion

Isolation of casein from cow's milk. Casein is derived from cow's milk because, according to Park et al. (2007) content of casein in cow's milk is greater than the milk of goats [13]. Higher casein content (2.6%) compared with the content of goat's milk (2.4%) and men (0.4%) than that of cow's milk is more easily obtained. Isolation of casein begins with the mixing of milk and rennet at a temperature of 35 ºC. This is because enzymes work optimally at a temperature of 35-40 ºC. The first isolation of casein by mixing milk and rennet at a temperature of 35 ºC so that the mixing process can take place quickly, rennet will work evenly to form a precipitated. There is a protease enzyme in rennet which is in charge of deciding ties on casein. These enzymes are hydrolyze specific binding chymosin on kappa-casein milk, resulting in termination of the bond, the milk, kappa-casein acts as a stabilizer [14]. After the activity is destroyed by chymosin, coagulation will occur so that the casein can be precipitated and separated by decantation and filtered. The resulting sediment is stored in the fridge and closed the meeting, because it is easily attacked by bacteria.

This method is made simpler in isolating casein.The result in a larger scale production is faster inproducing casein. Casein is obtained to be tested dry weight. Casein was obtained in the wet weight of the final stage of the process of decantation while filtered. Weighed 1 gram wet weight and then put in the oven to 110 ºC. Weighing is done once every 1 hour 3 data to obtain a constant weight.

Extraction of propolis. At this stage of propolis extraction method used is a method of maceration. Maceration is one of the extraction method used for materials that are not heat resistant. This method of soaking the material with a specific solvent and a period of time at room temperature. In this study the solvent used is ethanol 96%, which is semi-polar, so that the active compounds with different polarity is expected to be extracted perfectly. Maceration method was used while do the stirring (mixing) so that the active compounds are extracted and a lot faster. After maceration, 96% ethanol extract of propolis (EEP 96%) seen in Figure 4.1, resulting in a resin and wax (wax) is also participating extracted that were deemed to be a lot of impurities in the extract of propolis, so one needs to separate in order to obtain a more pure propolis without impurities. On the separation of wax takes the optimal separation.

To achieve optimal separation between propolis with its impurities, performed by diluting using distilled water. Dilution were done using distilled water until the concentration of ethanol 70% (EEP 70%). Ethanol 70% is the optimum conditions for extracting propolis bioactive extract of propolis [10]. The purpose of this separation is that the solubility of wax on decreases, so the ethanol contained in propolis wax will precipitate and then separated by filtered. After that the bottom layer of thick white candle that is, separated using a vacuum filter. Propolis is already separated from the wax and resin. Then propolis dilluted with glycerol (food grade) as a solvent that is safe for human consumption. Later stages of propolis has evaporated to remove ethanol and water levels. Propolis will look viscous after a process of evaporation because there the a solvent glycerol propolis. Ethanol was removed for safe human consump tion. Water content was removed to a more pure propolis and improve quality.

Encapsulation of propolis extract with Casein. In this process the solution used for the coating process is a phosphate buffer. Phosphate buffer pH serves to maintain and to reshape the bridge of calcium phosphate, with the addition of $CaCl_2$ in stages so that casein can coated propolis. At the time of the addition of $CaCl_2$ pH is also maintained by adding a solution of 0.1 M HCl, 0.1 M KOH. After the coating of casein with propolis, further to reduce the size of a nano particle tools used "High Pressure Ball Mill Homogenize". Samples will be destroyed by ground first, then with high pressure in the particle push and hit the ring in the instrument, so that a nano-sized particle distributions. In a large-scale manufacture of these devices suitable for use because in first running its use is 1 kg sample that can be used as nanoparticles. In the study conducted Semo et al. (2007), the process used to form the nano-size high-pressure centrifugation [15]. In the present study, performed using the High Pressure Ball Mill Homogenizer and the result does not affect the activity of the resulting product.

Encapsulation efficiency. Tests were performed using the spectrophotometric analysis of two parameters, namely the levels of polyphenols and flavonoids content. The method used to measure the polyphenols is a method follin-ciocalteau, in the sample when there are polyphe-nolic compounds will give a blue color after addition of reagent follin and absorbance was measured with a spectrophotometer, the results obtained compared with the standard test used, namely gallic acid.

For flavonoid analysis using aluminum chloride ($AlCl_3$), in the sample when there are flavonoid compounds will give a greenish yellow color after the addition of $AlCl_3$, the results are compared with the standard test used, namely quercetin. After the measurements performed calculations to determine the levels of each test. Here is the Table 1. spectrometry analysis results for these tests.

In Table 1 can be seen levels of total flavonoids of propolis 2.028.571,4 mg and total flavonoid content of 107,142.8 mg in supernatant ePCM. From these results can be calculated encapsulation efficiency. From the

Tabel 1. Spectrophotometric Assay Results

Sample	Content of Flavonoid (µg)
Propolis	2.028.571,4
Supernatant ePCM	107.142,8
Encapsulation efficiency	94,71%

experimental results, encapsulation efficiency of flavonoids by 94.71%.

According to Chen et al., a good encapsulation efficiency of at least 80%, because it shows the process do not eliminate the existing active substances.. In the study Semo et al. obtained 85% efficiency with the casein micelle coating to coated single compound, that is vitamin D2. In a previous study that Sahlan et. al (2012) obtained 93.9% efficiency using casein micelle coating with propolis "Cibubur" active ingredient. Sahlan et. al also have levels of flavonoids from propolis cibubur of 1846.15 ppm, while the results of my research of propolis flavonoid content of raw materials derived from the "Madu Pramuka" has levels of 2028.57 ppm. This research is very good for getting levels of flavonoids and flavonoid bioactive coating efficiency is higher than previous studies [8, 10, 15].

Nanoparticle size analysis. Measurement of nano particles using particle size analyzer (PSA). Particle measurements carried out to see the particle size of casein micelle nanopropolis with the coating. Measurements were made on the final product that is nanopropolis which is a result made into the size of the nano particle size using a high pressure ball mill homogenizer. In the test particle size is expected to form the nano-sized particles. The importance of the results obtained in the form of nano particles due to the nano-sized products, the drug delivery process to be more selective in maximal and specific areas of the body and minimize the occurrence of side effects [8].

From the results of particle measurements using the PSA, in samples taken twice nanopropolis measurements. In the first measurements of the samples had an average diameter of 75.7 nm, 86.3 nm of the largest and smallest measurement of 68.9 nm, while for the latter having an average diameter of 83.9 nm, 94.1 nm of the largest and smallest 72 nm. Based on these results, to produce nanoparticles (<1000 nm) [16]. Also affects the particle size to nano-delivery system can be developed when NePCM for the design of such supplements and other products consumed by the body, it is very advantageous because it is easily absorbed. To assure again that these nano-sized particles should be analyzed SEM (Scanning Electron Microscope) or TEM (Transmission electron microscopy), but because it is still constrained by the tools that have not been found in Indonesia, this analysis can not be done.

Figure 1. The Example of Accepted and Rejected Formulas. A and B are Rejected Formula and C is Accepted Formula

In the previous study showed that the particle size can be 316,1 nm. Particles to this size is still quite large compared with the results get. Particle-making process on research Sahlan et al. is sonication [10]. This technique uses a tool that makes sonicator nano-sized particles due to ultrasonic waves. Our other research is much smaller in size, so that the nano particles of a material which has a particle size smaller (nanometer) will be more easily absorbed by the body. High pressure ball mill homogenizertools prove the resulting particle size is smaller.

Production of the sunscreen cream. The creams was produced with 5 main component, oil based, water based, active sunscreen agent, emmolient, and emulsifier. Olive oil was used as oil based, because olive oil contain oleuropein that can reduced Reactive oxygen species (ROS) which can caused damaging the skin [17]. Rose water and aquadest was used as water based. TiO_2 and Propolis or Nanopropolis are used as sunscreen agent. Beeswax or carnauba wax and sorbitan monostearate were used as emulsifier. And isopropyl myristate as emolient. The first, the study focused on selection of the formula of them. Several formulas were accepted and several formulas were rejected. Figure 1 shows the example of rejected and accepted cream. 1A and 1B are the rejected formula, the sample 1A is too dry and sample 1B was not well mixed between oil based with other ingredients. The sample 1C is the example of accepted formula.

After optimizing the composition. The best formulation was 56.92% w/w of olive oil, 5.69% w/w of carnauba wax or beeswax, 4,39% w/w aquadest, 14-16% w/w nanopropolis, 10% w/w TiO_2, 4% w/w emmolient iso propyl myristat, and 5% w/w of sorbitan monostearate.

Stabillity of cream. The pH of the samples were monitored every 7 days for 28 days. The pH of the samples were stable in pH 5, there are no decreasing and increasing for all samples. The physic stabillity was analyzed by organoleptic which monitored the stability of shape, color, odor, and homogenity. The results from respondent showed that all physical stability of all sampels have maximal score for all parameters (data not shown).

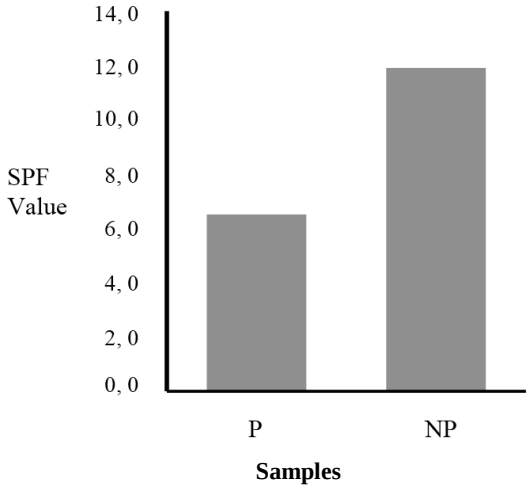

Figure 2. Comparison Photoprotection Activity between Propolis and Nanopropolis. P for Propolis, NP for Nanopropolis

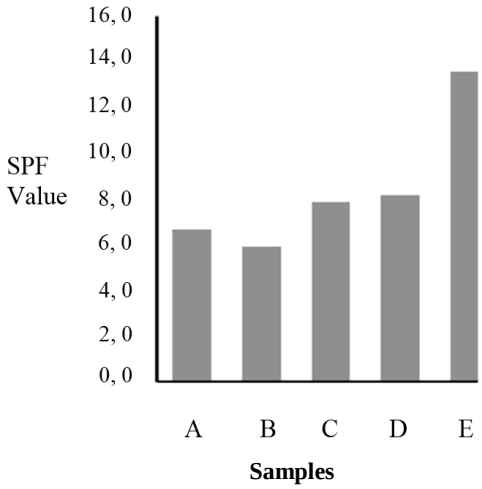

Figure 3. Comparison Photoprotection Activity between Nanopropolis and Nanocasein, Controls and also con Centration Nanopropolis. A for Cream bees wax without other Active Ingredients, B for Cream Carnauba wax and 14% of Nanocasein, C for Cream Beeswax and 14% of Nanocasein, D for Cream Beeswax and 14% of Nanopropolis, and E for Cream Beeswax and 16% of Nanopropolis

Photoprotection Acitivity Photoprotection activity of the creams monitored by SPF spectrophotometer Opto metric 290s. Photoprotection activity monitored for 6 sample which compare between sunscreen agent propolis and nanopropolis, and the influence of active agent concentrations and comparison photoprotection activities between carnauba wax and bees wax cream. Figure 2 shows that active agent of the encapsulated propolis have higher photoprotection activity compared with only propolis. The encapsulation process can protect the propolis from production process of cream and also from oxidation. It also might be the particle nanopropolis can spread well in the cream compare with propolis only.

Figure 3 shows the comparison of photoprotection acti vity of sunscreen active agent between nanopropolis and nano casein, the influence of nanopropolis concentration and comparison between beeswax and carnauba wax. The creams D and E have higher SPF value than cream A which mean that encapsulated propolis can improve photoprotection activity in sunscreen cream. The incre asing nanopropolis concentration also can improve the photoprotection activity (cream D containing 14% nano propolis and cream E was 16% of it). The cream C is the cream control that comparing between encapsulant and the encapsulated product in the cream D, the result shows that the encapsulated propolis by casein micelle have higher photoprotection activity compare with only casein micelle. The propolis can improve photoprotec- tion of casein micelle and this results showed that casein also have photoprotection activity.

The carnauba wax and beeswax are common emulsifier that used in sunscreen cream, they also have photoprotec tion activity. In this study we also compare the photoprotection activity between carnauba wax and beeswax. Figure 3B cream for carnauba wax and 3C for beeswax, the result shows that beeswax have better photoprotection activity compare with carnauba wax.

4. Conclusion

Propolis extracts able to encapsulated casein micelle. Encapsulation efficiency of propolis by the casein micelle about 94.71% for their flavonoids compound. Nanopropolis particle measurements have an average diameter of about 80 nm. As the sunscreen agent the encapsulation process by casein micelle to propolis improved the sun protection factor value of the cream.

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