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Characteristic Evaluation of Various Formulations of Anti-Aging Cream from Carotenoid Extract of Bacterial Symbiont *Virgibacillus salarius* Strain 19.PP.Sc1.6

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Abstract: Premature aging can be triggered by free radicals from UV rays, since exposure to these rays can cause the skin to experience oxidative stress. Oxidative stress induces intracellular DNA damage, protein denaturation, and lipid peroxidation that lead to cell death. However, cell death can be prevented with antioxidants such as carotenoids, which are among the potential natural compounds for its treatment. Sources of carotenoids include microbial symbionts associated with *Sinularia* sp., one of which is the bacterium *Virgibacillus salarius* strain 19.PP.Sc1.6, a carotenoid-producing bacteria. This study aims to explore the utilization of carotenoids from the bacterium *V. salarius* strain 19.PP.Sc1.6 for the preparation of anti-aging creams. Furthermore, the method employed three formulations (vs, ow, and wo) containing different types of cream tested for stability, and antioxidant and sunscreen abilities. The results obtained established that the carotenoid extract from *V. salarius* strain 19.PP.Sc1.6 was more stable in the cream vs. the oil-in-water type cream with an anionic emulsifier.

Keywords: cream; carotenoids; antioxidants; sunscreen; bacterial symbiont

1. Introduction

Premature aging is a degenerative disease characterized by dry, wrinkled, rough skin and black spots [1]. Two factors trigger premature aging, namely internal factors such as stress, endurance, hormonal changes, and health as well as external factors including ultraviolet rays and free radicals. Free radicals are oxygen-containing molecules whose atomic arrangement is unstable and, hence, undergo chain reactions that can occur in the body and lead to continuous damage [2].

Free radicals are very reactive and dangerous substances that cause damage to the tissues of the body which may lead to the development of various diseases in old age [3]. However, free radicals are possible to overcome by using antioxidants [4] which stop

chain reactions triggered by free radicals by donating electrons to the unstable molecules. Examples of antioxidant compounds include carotenoids, vitamin C, and vitamin E [5].

Carotenoids are compounds proven to be potential antioxidants [6–10] as they contain a long-chain isoprene compound with a conjugated double bond [5,7,11]. The conjugated double bond removes singlet oxygen and deactivates other free radicals occurring through the electron transfer process [12,13]. Subsequently, compounds that have conjugated double bonds can be found in terrestrial and marine organisms. Compounds from marine organisms have unique characteristics and several discoveries about new bioactive compounds for antibiotics, anti-cancer, pharmaceuticals, cosmetics, enzymes, and pigments have been obtained from many coral ecosystems, especially soft corals.

Massive utilization of soft corals should not be used, since it will cause damage to the ecosystem. The bacterial symbiont associated with soft coral could become a potential alternative which produces carotenoids as a source of antioxidants [14,15]. Cosmetic formulations that can be applied effortlessly and comfortably are required when using carotenoid compounds. Hence, the cosmetic formulation that is preferred is in the form of cream. Cream is the selected semi-solid preparation easily spread evenly on the skin, is non-sticky, and is easy to clean so it is comfortable to use by consumers. The use of symbiont bacteria as a source of cosmetic raw materials is the latest breakthrough that needs to be developed.

2. Materials and Methods

2.1. Materials and Tools

The bacterial symbiont *V. salarius* strain 19.PP.Sc1.6 isolated from *Sinularia* sp. originated from Panjang Island, Jepara, Central Java, Indonesia; the specific point is at the coordinates of "6°34′37.35″ S", "110°37′52.01″ E". Materials required include methanol (analytical grade) purchased from Merck (Darmstadt, Germany); 2,2-Diphenyl-1-picrylhydrazyl (DPPH) from Sigma-Aldrich Co. (St. Louis, MO, USA). Other ingredients used in the cream formulations such as stearic acid, glycerin, Na tetraborate, triethanolamine (TEA), lanolin, sunflower oil, beeswax, cetyl alcohol, span 80, glycerol monostearate, and Tween 80 were purchased from Merck (Darmstadt, Germany). The instruments used were an UV-vis Shimadzu 1240 spectrophotometer (Kyoto, Japan), Brookfield LVDV-I Prime Viscometer (Toronto, ON, Canada), pH meter digital USB Phs 3c 3d Rohs, centrifuge IEC Centra MP4R (Thermo Electron, Waltham, MA, USA), and orbital shaker Daihan SHO 2D (Wonju, Korea).

2.2. Isolation of Bacterial Symbiont of Sinularia sp.

The *Silunaria* sp. tissues were placed into sterile petri dishes that were partially filled with sterilized sea water. The surfaces of the soft coral tissues were cut away, and only the inside of the sample was used for microbial isolation. For bacterial isolation, a series of dilutions was conducted for all samples. From each petri dish, 10 mL of the sample was collected with a sterile pipette and placed into a flask containing 90 mL of sterilized sea water to obtain a 10^{-1} dilution. From the 10^{-1} dilution, 1 mL was moved into a tube containing 9 mL of sterile sea water to obtain a 10^{-2} dilution. This dilution process was repeated until a 10^{-5} dilution was obtained. A sample (1 mL) of each dilution series was collected and placed into a sterile petri dish containing Zobell 2216E agar medium (Himedia, Mumbai, India) for bacterial isolation. Then, the petri dishes were incubated at 30 °C for 2 days. Yellow-colored bacterial colonies that grow on the surface of the agar media were separated by a streak method to obtain pure bacterial strains.

2.3. Bacterial Symbiont Culture

The bacteria *V. salarius* strain 19.PP.Sc1.6 [16] was inoculated by transferring 1 mL of the isolate into 2 L of sterile Zobell Marine Broth 2216 (Himedia, Mumbai, India). It was then placed on the shaker at 100 rpm at 27 °C until the media color turned yellow (±7 days). Subsequently, the symbiotic bacteria that had produced carotenoids were isolated and

centrifuged for 15 min at 6000 rpm, after which the pellets separated and then filtered using Whatman filter paper No 1 (Maidstone, UK).

2.4. Carotenoid Extraction

Extraction of carotenoids using the fast of maceration method carried out in a dark room. Methanol (analytical grade) purchased from Merck (Darmstadt, Germany) was added to the pellet culture and then centrifuged for 10 min at 6000 rpm. Subsequently, a filtrate containing carotenoids and pellets in the form of a bacterial mass was obtained, filtered with a Wahtman No 1 filter paper, evaporated, and dried using N gas [14,15].

2.5. Cream Formulations

The formula employed three formulations with different types of cream, namely vs, ow, and wo [16–18]. The formula used is as shown in the Table 1.

Table 1. Cream formulas of carotenoid extract *V. salarius* strain 19.PP.Sc1.6.

Formula 1: Cream vs		**Formula 2: Cream ow**		**Formula 3: Cream wo**	
Stearate Acid	14%	Sunflower oil	6.5%	Lanolin	10.2%
Glicerin	10%	Glycerol monostearate	2%	Sunflower oil	40%
Na tetraborate	0.25%	Cetyl alcohol	3%	Beeswax	5%
TEA	1%	Tween 80	6%	Cetyl alcohol	5%
Carotenoid extract	0.44%	Carotenoid extract	0.44%	Span 80	3%
Aqua ad	100%	Aqua ad	100%	Carotenoid extract	0.44
				Aqua ad	100%

Note: The concentration of carotenoid extract used from the IC_{50} × 100 value is the carotenoid extract of *V. salarius* strain 19.PP.Sc1.6.

2.5.1. Formula 1: Cream vs

We melted the stearic acid and glycerin in a bowl. Na tetraborate and TEA were dissolved in a little water, then put in a solution of steric acid and glycerin. Then, we stirred and homogenized, and added a little water so that the results are good. The final step was to add the carotenoid extract and stir until evenly distributed.

2.5.2. Formula 2: Cream ow

First, the glycerol monostearate and cetyl alcohol were melted in cup 1. Sunflower and Tween 80 were mixed in cup 2. After that, we placed the mixture in cup 2 into cup 1. This was stirred until homogeneous with a little hot water. Finally, we added the carotenoid extract and stirred until homogeneous.

2.5.3. Formula 3: Cream wo

The first step was to melt the lanolin, sunflower, and span 80 in a cup 1. Then, we melted the beeswax and cetyl alcohol in a cup 2. We mixed cup 2 into cup 1 while stirring and homogenized it with a little hot water. Finally, we added carotenoid extract and homogeneous.

2.6. Cream Evaluation

The characteristics of three cream formulations were evaluated; the initial evaluation carried out was an organoleptic test. In organoleptic tests, observations include changes in color, odor (rancidity), and feel. In the organoleptic test, 25 people were used as respondents, consisting of 15 trained and 10 untrained people. The following test was homogeneity, which is accomplished by applying the cream to glass plates and observing the evenness of the color, whether it is even or not. Subsequently, in the pH test, the cream was deposited into a glass beaker, and then the pH measurement was done using a pH meter that had previously been calibrated with a Dappar standard (pH of 4 and pH 7). The scatter power

test was carried out on transparent glass placed on graph paper and 0.5 g of cream was placed on the glass and then covered with another transparent glass and left for ±5 s to obtain the diameter of the area formed. Then, different weights of 50, 100, 150, 200, 250, 300, 350, 400, and 450 g were placed on the transparent glass and the diameter of the area formed was observed. The specifications state that the cream should spread easily and evenly. Meanwhile, the cream viscosity test was determined using a Brookfield LVDV-I Prime Viscometer. An adhesion test was carried out with a glass object marked as 4 × 2.5 cm, then as much as 0.25 g of cream was placed at the midpoint of the area and covered with other glass objects. Therefore, a load of 1 kg was placed for 5 min, then the two glass objects that had been attached were mounted on the test equipment which was given a load of 80 g. After this, the time needed to separate the two glass objects was noted. Observations for all tests were carried out weekly for 5 weeks.

2.7. Measurement of Antioxidant Activity

Creams dissolved in methanol were prepared into a series of concentrations. Hence, a total of 3 mL of the sample was diluted with 1 mL of 0.1 mM DPPH solution and allowed to stand for 30 min. On settling, the solution was homogenized and its absorption was measured using a spectrophotometer at a wavelength of 517 nm. The absorbance of the control solution, that is, DPPH without test solution, was also read. Furthermore, the amount of antioxidant activity was measured using the following equation [19]:

$$\% \text{ Inhibitory } = \frac{[A517]_{control} - [A517]_{sample}}{[A517]_{control}} \times 100\% \quad (1)$$

where *control* = stock solution of DPPH.

2.8. Measurement of Sun Protection Factor (SPF)

Measurement of the Sun Protection Factor (SPF) value was done in vitro. The SPF test was performed to determine the sunscreen activity of the cream using a UV-Vis spectrophotometer. Each cream, weighing 0.1 g, was dissolved in 100 mL of methanol in a volumetric flask and filtered using Whatman No. 1. A total of 3 mL of the solution was put into a cuvette to measure its absorbance using a UV-Vis spectrophotometer. Subsequently, determination of the SPF value using the UV-Vis spectrophotometer was known from the characteristics of cream uptake at wavelengths of 290 to 320 nm with intervals of 5 nm. Calculation of SPF value uses the following equation [20,21]:

$$\text{SPF } = \text{ CV } \times \sum_{290}^{320} EE(\lambda) \times I(\lambda) \times \text{abs}(\lambda) \quad (2)$$

where:

CV = correction factor;
EE = Spectrum of erythema effect;
I = spectrum of sun's intensity;
abs = Absorbance of sample.

3. Results

3.1. Bacteria Culture and Carotenoid Extract

V. salarius strain 19.PP.Sc1.6 is a sample of bacterial symbiont associated with a soft coral *Sinularia* sp. [16]. Bacteria culture and carotenoid extract of *V. salarius* strain 19.PP.Sc1.6 are shown in Figure 1.

(**a**) (**b**)

Figure 1. (**a**) Bacterial culture and (**b**) carotenoid extract of *V. salarius* strain 19 PPSc1.6.

3.2. Cream Evaluation

Photoaging creams were prepared using three formulations with different types of creams (Figure 2). The first formulation was cream vs, a type of oil-in-water cream with an anionic emulsifier, the second is cream ow, a type of oil-in-water cream with a nonionic emulsifier [22], and the third is cream wo, a water-in-oil cream. The results of the evaluated cream preparations are shown in Table 2. The stability test of this study was carried out at room temperature; the results are shown in Table 3 and Figure 3.

(**a**) (**b**) (**c**)

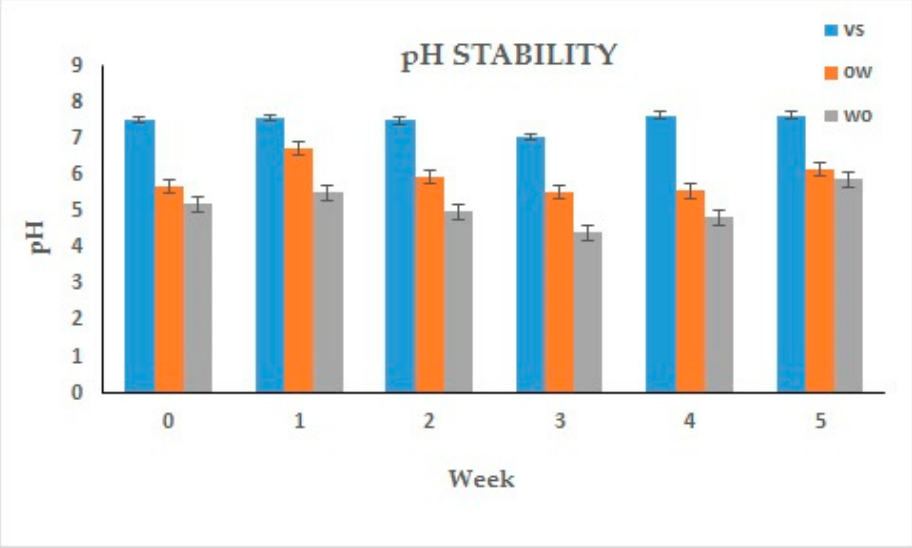
Figure 2. vs (**a**), ow (**b**), and wo (**c**) cream.

Table 2. Evaluation of different parameters of cream formulations.

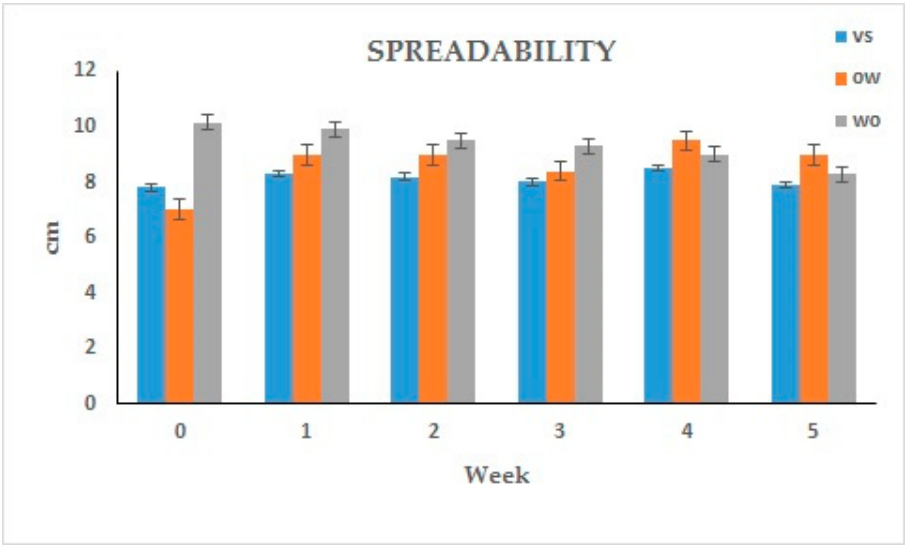
Cream Evaluation	Formula 1. Cream vs	Formula 2 Cream ow	Formula 3. Cream wo
Color	White	White	Yellowish
Smell	Odorless	Odorless	Odorless
Feel	Soft	Soft	Soft
Homogeneity	Homogeneous	Homogeneous	Homogeneous
pH	6.5	5.7	5.2
Spreadability (cm)	7.8	10.2	7
Viscosity (cps)	11,301	5642	11,506
Stickiness (sec)	0.71	0.80	0.80

Table 3. Organoleptic stability of cream formulation.

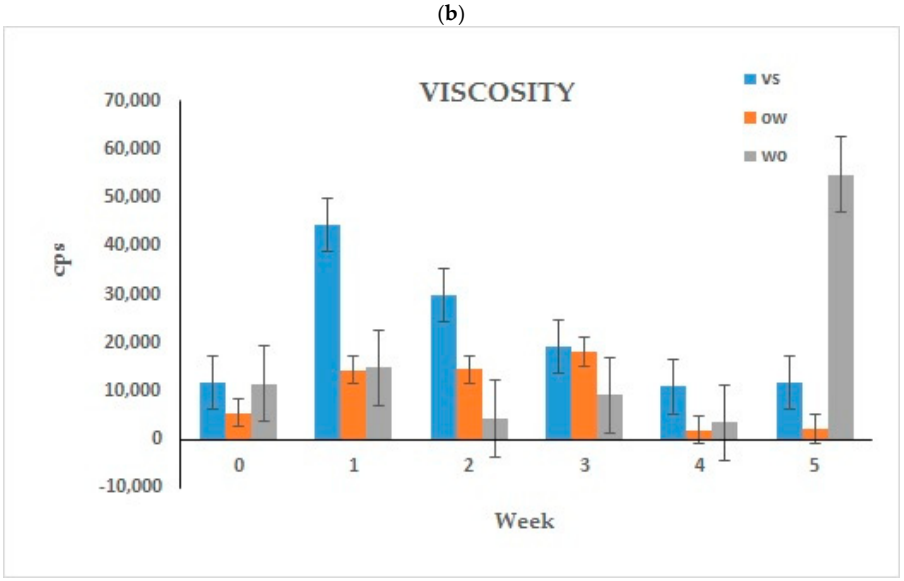
Cream Evaluation	Week	Formula 1. Cream vs	Formula 2 Cream ow	Formula 3. Cream wo
Color	1	White	White	Yellowish
	2	White	White	Yellowish
	3	White	White	Yellowish
	4	White	Yellowish white	Yellowish
	5	White	Yellowish white	Yellowish
Smell	1	Odorless	Odorless	Odorless
	2	Odorless	Odorless	Odorless
	3	Odorless	Odorless	Odorless
	4	Odorless	Odorless	Odorless
	5	Odorless	Odorless	Odorless
Feel	1	Soft	Soft	Soft
	2	Soft	Soft	Soft
	3	Soft	Soft	Soft
	4	Soft	Soft	Soft
	5	Soft	Soft	Soft
Homogeneity	1	Homogeneous	Homogeneous	homogeneous
	2	Homogeneous	Homogeneous	homogeneous
	3	Homogeneous	Homogeneous	Homogeneous
	4	Homogeneous	Homogeneous	2 phases
	5	Homogeneous	2 phases	2 phases



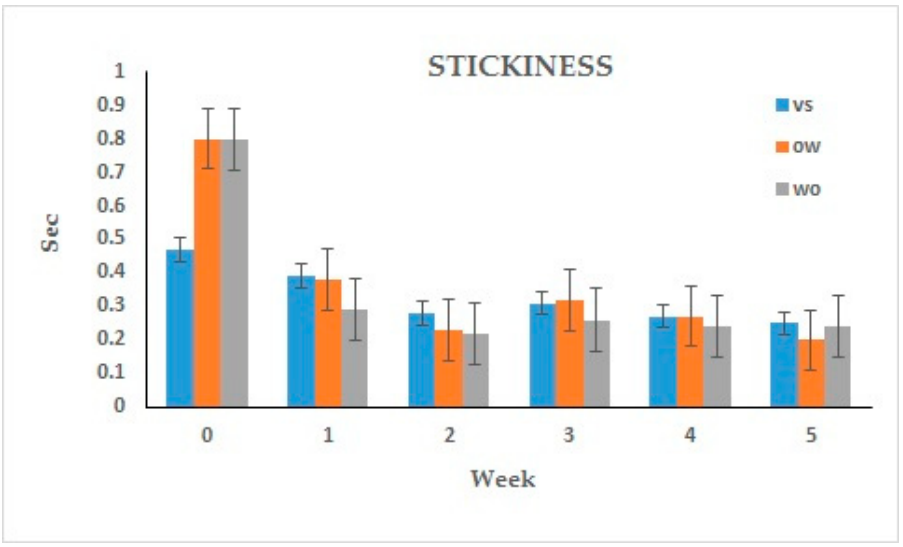
(**a**)



(**b**)



(**c**)

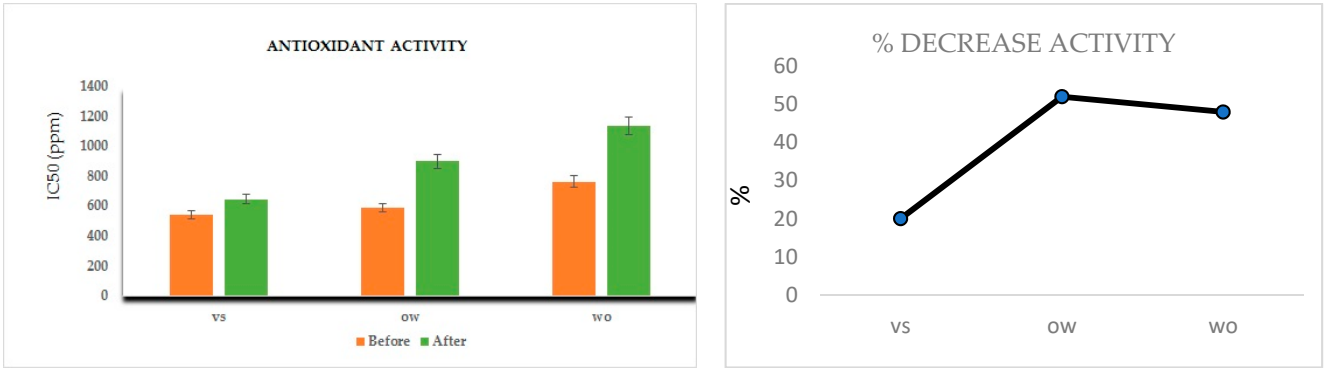


(**d**)

Figure 3. Evaluation of cream stability (**a**) pH; (**b**) spreadability; (**c**) viscosity; (**d**) stickiness.

3.3. Antioxidant Activity

The test for antioxidant activity was done before and after storage for five weeks. The results of the antioxidant activity are shown in Figure 4.

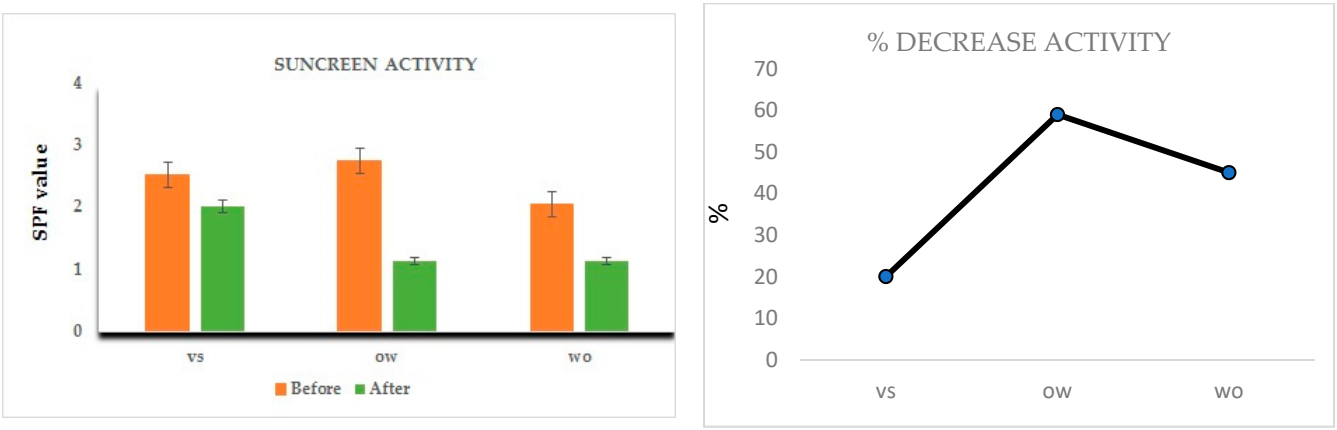


(**a**) (**b**)

Figure 4. Antioxidant activity of the cream before and after (**a**) storage and % decrease activity (**b**).

3.4. Measuring of Sun Protector Factor (SPF)

The sunscreen activity in the cream was measured by observing the SPF value. Therefore, the SPF measurement results before and after storage are shown in Figure 5.



(**a**) (**b**)

Figure 5. Sunscreen activity of the cream before and after storage (**a**) and % decrease activity (**b**).

4. Discussion

4.1. Cream Evaluation

On evaluation, the creams possessed the appropriate specifications for cream preparations as observed in the organoleptic test. Similarly, the results of the pH measurement also met the specifications for skin pH, as cream preparations must have a pH that matches the pH of the skin in the range of 4.5–6.5 [23–25]. Spread also affects the nature of the cream when used as a topical preparation because the greater the spread area, the greater the surface area of the skin that comes into contact with the cream, and this ensures easy distribution of active substances [1]. The viscosity test results of the three creams according to the specifications for semi-solid preparations are 4000–40,000 cps [26]. Furthermore, stickiness is related to the duration of time the cream stays in contact with the skin, and the longer the cream sticks to the skin, the more effective the active substance will be [4]. The results of the three creams showed almost similar stickiness.

The stability results for the third organoleptic test indicated that the cream was stable because there were no changes in color, odor, or texture. Furthermore, the homogeneity test is essential because it is related to the effectiveness of the cream at the same level

of use. Hence, the active substance will be the same with every use if the preparation is homogenous [27]. Cream is considered to be homogeneous when there are no clumps seen physically in it [28]. For ow and wo creams, a two-phase separation occurred at weeks four and five, and this separation occurred due to the possibility of instability of the active substance and a decrease in the emulsifier [29].

For the third pH test, the cream was also stable in the skin pH range, and therefore it is safe to use. Creams with extremely alkaline pH cause scaly skin while an extremely acidic pH will cause skin irritation [30,31]. Therefore, monitoring the pH of the preparation is very important to ensure the stability of the emulsion as significant pH changes indicate a chemical reaction that can give an idea of product instability [32]. The spread of the three formulations increased during storage due to a decrease in the viscosity of the cream. Decreases in fluid resistance to flow increase the spread of cream [4]. The viscosity of the three creams had a similar tendency to decrease, as a decrease in viscosity can occur due to a decrease in emulsion stability over time. The viscosity test is also strongly influenced by viscosity as the greater the viscosity, the longer the adhesion. In addition, if the viscosity decreases, the sticking power will follow as well [23].

ANOVA analysis showed that the level of significance was >0.05 for the pH, viscosity, and stickiness, which indicates that storage time has no significant effect on pH, viscosity, or stickiness. The statistical results indicate that the resulting cream formula is quite stable in storage for five weeks. Furthermore, the ANOVA analysis on spreadability showed that there was no significant difference between the weekly variants; however, there was a significant difference in the cream variant, hence, the variation between the vs and wo formulas was significantly different.

4.2. Antioxidant Activity

The oil-in-water cream had better antioxidant activity compared to the water-in-oil type. These results are similar to the results obtained by previous research [17] that stated that carotenoids are more stable in the oil-in-water cream type. Furthermore, of the two types of oil-in-water, namely vs and ow, vs had better antioxidant activity, as creams with an anionic emulsifier had better antioxidant activity than those with nonionic emulsifiers [22]. The reducing effect of the active ingredient on DPPH may also be influenced by stearic acid used as an emulsifier in cream vs. Stearic acid can also help carotenoids in reducing DPPH.

In this study, cream storage was also carried out for five weeks to compare antioxidant activity before and after storage. The results obtained indicated that antioxidant activity decreased during storage. In addition, carotenoids used as active ingredients in cream preparations had a decreased number [17], which caused a reduction in antioxidant activity. Statistical data with ANOVA showed a level of significance of >0.05, which indicates that storage time has no significant effect on the value of the antioxidant activity.

4.3. Measuring of Sun Protector Factor (SPF)

By definition, cream is categorized as having minimum sun protection if it has an SPF value of 2–4 [32]. The three creams before storage had sunscreen protection; however, after five weeks of storage, the sunscreen protection ability was lost because the SPF value was below 2. The decrease in the SPF value might be influenced by a decrease in carotenoid levels when it is the active ingredient since carotenoids are susceptible to oxidation during storage [33]. Furthermore, when degradation occurs, the biological activity will be reduced [13,34]. Statistical results show a significance value of 0.109 > 0.05, which means that the storage time does not result in a significant difference in the SPF value.

5. Conclusions

V. salarius strain 19.PP.Sc1.6 is a bacterium associated with *Sinularia* sp. that produces carotenoids with antioxidant and anti-aging properties. This research utilized carotenoids from *V. salarius* strain 19.PP.Sc1.6 for cream preparation and the results showed that these carotenoids were stable in the oil-in-water type with an anionic emulsifier (formulation vs). Furthermore,

the antioxidant activity and sunscreen protection were the highest in formulation vs, indicating that the stability of carotenoids affects the antioxidant and sunscreen activity.

Utilization of symbiotic microorganisms, especially bacteria, needs to be developed for cosmetic raw materials. The advantage that can be obtained is that the growth of bacteria is fast and does not over-exploit the ecosystem, therefore it does not damage the environment.

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Data Availability Statement: The data presented in this study are available on request from the corresponding author.

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Conflicts of Interest: The authors declare no conflict of interest.

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