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Effect of astaxanthin-enriched virgin coconut oil concentration on lip balm properties and sun protector factor (SPF) activity

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Abstract

Background: Our previous research successfully extracted astaxanthin from fermented Acetes shrimp (cincalok) using virgin coconut oil (VCO), resulting in astaxanthin-enriched VCO (VCOA). This innovative approach demonstrates the feasibility of incorporating VCOA into cosmetic preparations, in particular, its application in lip balm formulations.

Objective: This study aimed to formulate a lip balm incorporating VCOA in combination with tengkawang butter.

Method: The physical properties and sunscreen activity of the lip balm were evaluated as a function of storage duration at room temperature. The study examined the influence of varying concentrations and compositions of astaxanthin-enriched virgin coconut oil (VCOA) and illipe fat on the formulation. Key parameters assessed included homogeneity, pH stability, color stability, and melting point.

Results: The test results indicated that a homogeneous lip balm was obtained when a high concentration of VCOA (15-30% w/w) was used and was stable for 28 days of storage. The acidity level (pH: 4.56-6.69) and melting point of the lip balm (50–67°C) have met the Indonesian National Standard (SNI). The presence of VCOA causes the lip balm to be yellow to orange. The resulting lip balm shows a sun protection factor (SPF: 51.1674 $\pm$ 0.0364) value with an ultra-protection category.

Conclusion: In this study, astaxanthin was extracted from cincalok using VCO to produce VCOA, which was directly applied as a lip balm ingredient. The application of VCOA at the highest concentration (30%) yielded lip balm with excellent homogeneity, stable pH and melting point in accordance with SNI standards, the most pronounced color, and the highest SPF value.

Keywords: astaxanthin, cincalok, cosmetic, lip balm

1. Introduction

Cincalok is a typical West Kalimantan food that is based on research by Mauludia *et al*. (2021), contains high astaxanthin, which is 1.47 mg/100 g wet weight. Furthermore, Rahmalia *et al*. (2022) reported that cincalok oil contains 21.70% palmitic acid, 10.99% docosahexaenoic acid (DHA), and 10.33% eicosatetraenoic acid (EPA). Cincalok oil also contains astaxanthin at 0.38 ± 0.02 mg/L of oil. Palmitic acid is a cosmetic ingredient that is commonly used as a cleaning agent and emollient. DHA and EPA, which are very abundant in the lip epithelium, can have an antioxidant role and thus can protect the lips from free radicals (Al Mamun *et al*., 2020).

Astaxanthin is a natural carotenoid compound that has many functions related to antioxidant and anti-inflammatory activities. Astaxanthin can be obtained through extraction methods. Prayitno *et al*. (2022) and Fitriani *et al*. (2023) have extracted astaxanthin using vegetable oil solvents and produced the highest yield when virgin coconut oil (VCO) was used as a solvent. The extraction results obtained are called virgin coconut oil enriched with astaxanthin (VCOA). Astaxanthin in VCOA has excellent stability to heat and UV rays (Prayitno *et al*., 2024). These advantages of VCOA make it potentially further processed as a nutritious food supplement or cosmetic raw material.

The cosmetic product that is the focus of this study is lip balm, a lip moisturizer product. Lip balm products are widely used because their formulations contain active ingredients that can protect the lips from exposure to sunlight and weather conditions. Lip balm formulation using VCO has been carried out by several researchers and has been reported to have many advantages, including excellent emollient properties; high antioxidant, antimicrobial, and anti-inflammatory content; and a light and easily absorbed texture. The saturated fatty acid content in VCO can also help increase the stability of lip balm formulations, prevent oxidation, and extend the shelf life of the product. These properties ensure that lip balm remains effective and safe to use for a longer period (Kusrini *et al*., 2020; Nareswari *et al*., 2022; Siahaan *et al*., 2022). In addition to the biodiversity of coconut trees that can produce VCO, Indonesia also has endemic flora that has great potential to be used as cosmetic ingredients, namely tengkawang. It is a group of trees from the genus *Shorea,* which belongs to the *Dipterocarpaceae* family. Tengkawang seeds contain vegetable fats that are rich in essential fatty acids such as myristic acid, palmitic acid, stearic acid, oleic acid, and linoleic acid. This fat has moisturizing properties that can be utilized in the cosmetics industry, such as in the making of hand and body lotion and lip balm (Gusti & Waluyo, 2011).

This study aims to use VCOA combined with tengkawang fat in formulating lip balm. The parameters that have been studied are the effects of the concentration or composition of VCOA and tengkawang fat in lip balm preparations on physical properties such as homogeneity, pH stability, color stability, and melting point. Therefore, lip balm preparations also require sunscreen activity to protect the lips from UV rays (Sabzevari *et al*., 2021); sunscreen activity tests have also been carried out based on the sun protector factor (SPF) value.

2. Method

2.1. Tools and materials

The materials used were distilled water (H_2O, Cleo), oxalic acid dihydrate ($C_2H_2O_4.2H_2O$, Merck), acetone (C_3H_6O, Merck), beeswax, dried cincalok, candelilla wax, ethanol (C_2H_6O, Merck), ethyl acetate ($C_4H_8O_2$, Smart Lab), pp indicator, buffer solution 7, buffer solution 4, tengkawang fat (Aracia), sodium hydroxide (NaOH), n-hexane (C_6H_{14}, Smart Lab), propylene glycol, strawberry fragrance oil, and virgin coconut oil (Arcia). The tools used were stirring rod, blender, weighing bottle, bulb, burette, colorimeter (WR10QC portable, China), glass funnel, Erlenmeyer, beaker, hotplate, glass preparation, measuring flask, magnetic stirrer, melting point (SMP10, USA), capillary tube, measuring pipette, dropper pipette, pH meter (Hanna instruments, Italy), spatula, UV-Vis spectrophotometer (Orion Aquamate 8100, US), sonication (Delta D68H), test tube, vortex, and lip balm container.

2.2. Extraction and determination of astaxanthin content

Astaxanthin extraction was carried out by adopting the method of Nurbaeti *et al*. (2021). Dried cincalok was added to VCO oil (the ratio of dried cincalok and VCO was 1:2 w/v), then blended for 5 minutes, rested for 5 minutes, and then blended again for 5 minutes so that the total blending time was 30 minutes. The calculation of the astaxanthin content contained in VCOA was carried out by adopting the procedure of Fitriani *et al*. (2023). The astaxanthin content contained in VCOA was determined using a calibration curve of standard astaxanthin. VCOA was pipetted as much as 1 mL, and acetone was added in a 5 mL measuring flask to the limit mark. The mixture was shaken until homogeneous; the absorbance was read at the maximum wavelength (477 nm). This process was repeated 3 times.

2.3. Water content and free fatty acid test of VCOA

The water content test of VCOA and tengkawang butter was carried out by weighing 1 gram and placing it in a weighing bottle of known mass. Then, the weighing bottle containing the sample was heated in an oven on a heater, the weighing bottle was cooled in a desiccator for 30 minutes. The weighing bottle that had cooled was then weighed to obtain its mass. Each treatment was repeated in triplicate (SNI 7381:2008).

The free fatty acid test was carried out by weighing 1 g of sample, then putting it into an Erlenmeyer flask and adding 5 ml of ethanol. After that, 3 drops of pp indicator were added and then titrated with a NaOH solution that had been previously standardized using oxalic acid until the endpoint was pink. The free fatty acid number was calculated based on SNI 7381:2008, and measurements were carried out in triplicate.

2.4. Lip balm formulation

The manufacture of lip balm using VCOA was carried out by modifying the methods of Limanda *et al*. (2019) and Desnita *et al*. (2022). Base A was put into a beaker of glass and melted on a hot plate stirrer at a temperature of 80°C. Base B was heated over a water bath and added to base A stirred until homogeneous. After base A and base B were homogeneous, base C was added until homogeneous, then put into a prepared lip balm container and solidified. The lip balm formulation can be seen in **Table 1**.

Table 1. Lip balm formulation

Ingredients	Concentration (%)							Utility
	F0	F1	F2	F3	F4	F5	F6	
Base A								
Candelilla wax	5	5	5	5	5	5	5	Stiffening agent
Beeswax	5	5	5	5	5	5	5	Stiffening agent
Tengkawang butter	30	25	20	15	10	5	0	Fat base
Base B								
VCOA	0	5	10	15	20	25	30	Oil and dye
Base C								
Strawberry fragrance oil	0.5	0.5	0.5	0.5	0.5	0.5	0.5	Fragrance
Propylene Glycol	5	5	5	5	5	5	5	Cosolvent

2.5. Physical properties and stability test of lip balm

The homogeneity test was carried out by applying the sample to a glass object and observing it visually. The purpose of this test is to identify whether there was a change in shape or inhomogeneity in the lip balm sample. If there was a change in shape or small grains, the observation indicated that the lip balm sample was not homogeneous. The pH test is carried out using a pH meter. The lip balm is weighed at 0.5 g and then dissolved in 5 ml of distilled water. The pH of the lip balm preparation was then measured by dipping the electrode of the pH meter into the solution until the pH appeared on the meter. The pH of the lip balm sample met the requirements if the pH value was close to or ranged from 4.5 to 6.5 (SNI 16-4769-1998).

The melting point test was carried out using a melting point apparatus. The lip balm was inserted into a capillary tube to a depth of 10 mm. Furthermore, the capillary tube was placed in the melting point apparatus in the correct position. The temperature at which the lip balm begins to melt was its melting point. Based on SNI 16-4769-1998, the melting point of lip balm ranges from 50 to 70°C. Lip balm color analysis used a measuring tool, namely the Konica Minolta CR-400 chroma meter. The chroma meter has three indicators, namely brightness level (L *), redness level (a *), and yellowness level (b *) (Azmin *et al*., 2020). Stability testing was carried out to see any changes in homogeneity, pH, melting point, and color of the lip balm preparation during storage on days 0, 7, 14, 21, and 28 at room temperature.

2.6. Determination of SPF value of lip balm preparations

Determination of the SPF value of lip balm preparations was carried out by modifying the method (Mbanga *et al*., 2014). Each lip balm preparation, F0, F1, F2, F3, F4, F5, and F6, was weighed at 0.5 g and dissolved with ethyl acetate solvent using a 5 ml measuring flask. After that, the solution was vortexed for 2 minutes and sonicated for 10 minutes. The solution was measured at a wavelength of 290-320 nm using a UV-Vis spectrophotometer. The test was carried out 3 times to obtain accurate values and calculated using the equation.

$$SPF = CF \times \Sigma 290\ 320\ EE\ (\lambda) \times I\ (\lambda) \times Abs\ (\lambda)$$

where:
CF = correction factor (10)
EE = erythema effect spectrum
I = light intensity spectrum
Abs = absorbance
The EE x I value is a constant

The categories were based on the SPF values calculated using the Mansur equation. Sunscreen protection was classified into five categories: minimal protection (SPF 2–4), moderate protection (SPF >4–6), extra protection (SPF >6–8), maximum protection (SPF >8–15), and ultra protection (SPF >15).

3. Result and discussion

3.1. Virgin coconut oil enriched with astaxanthin (VCOA)

Astaxanthin in cincalok is easier to extract than astaxanthin in fresh rebon shrimp due to the fermentation process. The cincalok fermentation process involves lactic acid, which can dissolve calcium salts in shrimp containing pigments. This process can release astaxanthin from protein and increase astaxanthin levels during extraction (Rahmalia *et al*., 2022). The use of VCO as a solvent is because VCO contains more medium-chain fatty acids (C6-C12) than long-chain fatty acids (C12-C18). Based on its fatty acid content, the polarity of VCO is higher than soybean oil or sunflower oil. Astaxanthin is a carotenoid with several ionone rings that show more polar properties than other carotenoids. Therefore, astaxanthin is more soluble in VCO (Fitriani *et al*., 2023; Prayitno *et al*., 2022). The extraction results obtained in the form of VCO enriched with astaxanthin from cincalok (VCOA) can be seen in **Figure 1**.

Figure 1. Virgin coconut oil enriched with astaxanthin (VCOA)

The orange color of VCOA indicates that the astaxanthin dye has been successfully extracted from VCO. The astaxanthin content in VCOA can be determined using UV-Vis spectrophotometry using the method conducted by Fitriani *et al*. (2023), with a maximum wavelength of standard astaxanthin in acetone of 477 nm. The astaxanthin content obtained in this study was 7.207 mg/L ± 0.4631, slightly lower than the results of Fitriani *et al*. (2023) using the glass bead vortex method. In addition to the difference in methods, the low level of astaxanthin extracted in this study was likely due to the difference in the cincalok samples used.

3.2. Water content and free fatty acids of VCOA

VCOA has a water content of 0.07 ± 0.036%. Based on SNI (2008), the water content of VCOA meets the standard, which is below 0.2%. Oil with a low water content indicates that it is of high quality. Meanwhile, high water content can cause the oil to undergo hydrolysis more easily, which can decompose it. Water content can also affect the free fatty acids contained in the oil. High water content can also cause high free fatty acid values. This phenomenon is because the reaction of free fatty acid formation in oil involves water (H_2O) which acts as a reactant (Ulfindrayani & A'yuni, 2018). Free fatty acids are a parameter that measures the amount of free fatty acids in fat after hydrolysis. Free fatty acids are formed from the degradation of triglycerides due to oil damage (Di Pietro *et al*., 2020; Wunderling *et al*., 2023). The ALB test was carried out using the acid-base titration method. The ALB value of VCOA is 1.52 ± 0.0642%. Based on SNI (2008) the ALB value of VCOA exceeds the standard because the ALB limit in oil is a maximum of 0.2%. However, according to IFOS (2014), the standard ALB value in animal oil is 1-7%. The high ALB value of VCOA calculated in this study is likely due to the high acidity of the fermentation product (Fitriani *et al*., 2023).

3.3. Lip balm from a combination of VCOA and tengkawang butter

Lip balm formulation with the addition of VCOA, replacing tengkawang butter produces a lip balm preparation that is yellow to orange in color (**Figure 2**). The components of making lip balm consist of candelilla wax, beeswax, tengkawang fat, VCOA, strawberry fragrance oil, and propylene glycol. The use of candelilla wax can provide a soft texture and shiny appearance (Gusti & Waluyo, 2011). Tengkawang fat can provide an oil coating that protects the skin's surface and prevents moisture loss. Several cosmetic products use astaxanthin as a natural dye for both skin care and makeup products. The more concentrated the VCOA added, the more intense the orange color of the lip balm will be. The lip balm preparations that have been made were tested for their physical properties, including pH, melting point, homogeneity, and color stability during 28 days of storage. In addition to testing physical properties, sunscreen tests were also carried out.

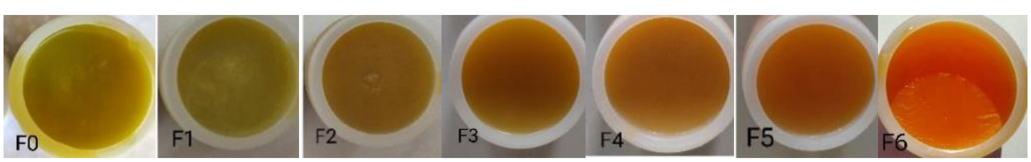

Figure 2. Lip balm preparations with varying VCOA concentration

Observation of lip balm homogeneity aims to ensure that the ingredients used are evenly mixed homogeneously (Limanda *et al*., 2019). Testing is carried out by applying lip balm to a glass slide and observing it visually. Based on **Figure 2** and **Table 2**, during 28 days of observation, in preparations F0, F1, and F2, there were small granules, indicating that the lip balm with this formulation was not homogeneous. This observation is because the lip balm components are not dispersed properly (Supartiningsih *et al*., 2021). Preparations F3, F4, F5, and F6 did not show any small granules after 28 days of observation. This indicates that the ingredients used in making lip balm are well mixed. This result also proves that VCO is able to disperse lip balm ingredients homogeneously.

Table 2. Results of homogeneity test of lip balm preparations

Formulation	Homogeneity				
	Day 1	Day 7	Day 14	Day 21	Day 28
F0	NH	NH	NH	NH	NH
F1	NH	NH	NH	NH	NH
F2	NH	NH	NH	NH	NH
F3	H	H	H	H	H
F4	H	H	H	H	H
F5	H	H	H	H	H
F6	H	H	H	H	H

H = homogeneous
NH = not homogeneous

The pH test aims to determine the acidity level of lip balm preparation, which can affect the stability of the active ingredients and ensure product safety for consumers when used. Based on the measurement results that have been carried out as presented in **Table 3**, the pH value of almost all lip balm preparations is in accordance with the physiological pH of the lip skin, which is 4.0-6.5 (Gunt & Ertel, 2023), except for the pH of the F0 preparation on the first day. However, after seven to 28 days, the pH of the F0 preparation decreased so that it was in the physiological pH range of the lip skin.

The F0 preparation does not contain VCOA. Preparations F1, F2, F3, F4, F5, and F6, which contain VCOA, have a relatively stable pH up to 28 days of storage. This is likely due to the difference in fatty acid composition between tengkawang oil and VCO. Tengkawang oil is rich in unsaturated fatty acids, especially oleic acid and linoleic acid, which are more susceptible to oxidation. Meanwhile, VCO contains mostly saturated fatty acids (especially lauric acid), which are more stable against oxidation. If there is moisture in the lip balm formulation, tengkawang oil, which has more unsaturated fatty acids, can undergo hydrolysis faster, producing free fatty acids that can contribute to a decrease in pH, making it more acidic. VCOA, with its low water content and the antimicrobial properties of lauric acid, is more resistant to hydrolysis.

Table 3. Degree of acidity (pH) during storage

Formulations	**pH**				
	Day 1	Day 7	Day 14	Day 21	Day 28
F0	6.69	6.02	6.18	6.21	4.56
F1	5.51	6.18	5.67	5.83	5.77
F2	5.80	6.05	5.85	5.95	5.73
F3	5.90	6.13	5.79	5.93	5.84
F4	5.94	6.01	5.75	5.90	5.84
F5	5.94	6.09	5.83	6.09	5.84
F6	6.36	6.04	5.31	5.45	5.35

The melting point serves as an indicator of the durability of the lip balm's texture during storage at higher temperatures (Puspita *et al*., 2023). The melting point test is an effective method to identify complex mixtures that do not have a specific melting point, such as in lip balm preparations (Susilo & Febriana, 2022). The component in lip balm, including fat, hardener concentration, and emollient, can influence the melting point of the preparations. **Table 4** presents the results of the melting point test of lip balm preparations during storage. In general, the melting point of the preparations is relatively the same and does not change significantly during storage. The melting point of the preparation ranges from 50 to 67°C. Based on the Indonesian National Standard (SNI) 16-4399-1996 concerning lipstick, which can also be used as a reference for lip balm, the recommended melting point ranges from 50 to 65°C. This range ensures that the lip balm remains stable at ambient temperature but melts easily when applied to the lips.

Table 4. Melting Point of lip balm preparations during storage

Formulations	**Melting point**				
	Day 1	**Day 7**	**Day 14**	**Day 21**	**Day 28**
F0	54	56	54	55	57
F1	50	56	56	56	57
F2	52	57	59	58	60
F3	55	62	62	60	62
F4	60	64	64	64	62
F5	61	65	65	67	65
F6	57	59	58	59	59

Adding color to lip balm preparations has several interests, both in terms of function, aesthetics, and product appeal. VCOA provides a yellow to orange color to lip balm preparations, which can enhance their visual appeal. Color tests were carried out using a chromameter with three indicators, namely brightness level (L*), redness level (a*), and yellowness level (b*). The test results are presented in **Table 5**. Based on the table, the brightness level of all preparations is relatively the same, ranging from 53.26 to 63.07, indicating that the lip balm in this study has a moderate brightness level. In the context of lip balm containing astaxanthin, an L value of around 53–63 can indicate a reddish orange color, which is in accordance with the characteristics of the natural pigment.

Table 5. Results of color stability measurements for 28 days

	Formulations	Color indicator				
		Day 1	**Day 7**	**Day 14**	**Day 21**	**Day 28**
L*	F0	57.74	58.37	59.78	58.52	59.07
	F1	59.05	59.10	58.83	59.13	59.14
	F2	57.07	57.06	56.75	57.27	57.59
	F3	60.54	63.07	61.82	62.86	60.61
	F4	56.49	53.26	58.09	54.81	56.41
	F5	58.08	58.60	59.66	59.91	60.52
	F6	55.38	55.81	57.73	57.62	56.17
a*	F0	-1.24	-1.26	-1.52	-1.39	-1.44
	F1	0.56	0.57	0.60	0.27	0.29
	F2	0.09	0.13	0.04	0.06	0.26
	F3	2.38	2.49	2.32	2.45	2.29
	F4	3.60	2.70	2.10	2.83	1.72
	F5	3.70	3.18	3.21	3.19	3.19
	F6	6.94	7.30	6.79	6.79	6.78
b*	F0	14.39	14.45	12.33	13.00	12.59
	F1	12.58	14.45	12.33	13.00	12.59
	F2	10.70	10.73	11.65	10.93	11.28
	F3	13.02	13.81	14.32	14.60	15.13
	F4	15.83	14.65	13.71	14.98	12.40
	F5	13.98	15.60	15.66	15.56	15.53
	F6	23.61	24.98	24.98	24.98	23.04

The higher the concentration of VCOA used, the higher the a* value increases, indicating a higher level of redness in the lip balm. In the F0 preparation, the a* value tends to be negative, meaning that the color of the lip balm is dominated by the green component compared to red. During storage, there was a change in value to -1.44, indicating a greater level of green color compared to the first day (-1.24). Preparations F1 and F2 showed CIELAB indicators for unstable a* values because this lip balm did not have satisfactory homogeneity. Preparations F3, F4, F5, and F6 had positive a* value ranging from 0.56 to 6.94 indicating that the lip balm was dominated by a reddish color, although with varying intensity. During storage, there was a decrease in the a* value, but it was not significant, possibly due to degradation of the astaxanthin pigment due to oxidation or interaction with other ingredients. The lip balm's color was dominated by yellow, as indicated by a positive b* value. On the first day, preparations F0, F1, F2, F3, F4, and F5 produced a softer yellow color compared to F6 (23.61, which indicates a yellow-orange color). Similar to the a* value, the b* value in all preparations experienced a slight decrease, which could be caused by degradation of the astaxanthin pigment during the storage process.

The SPF test aims to assess the effectiveness of a lip balm product to protect the lips from UV B rays from sun exposure (Cholis Endriyatno *et al*., 2024). Products containing sunscreen must have the ability to absorb light in the wavelength range of 290-320 nm. Sunscreen replaces the skin in absorbing UV B rays, thereby preventing sunburn and other skin damage. **Table 6** displays the SPF values and protection categories for the lip balm preparations. The table shows that the lip balm preparations F0, F1, F2, F3, F4, F5, and F6 are included in the ultra-protection category with the SPF values obtained. However, the F0 preparation has a lower SPF value than F1, F2, F3, F4, F5, and F6; this observation is an indication that the use of VCOA in lip balm preparations can increase the SPF value, although not significantly. The SPF value can be affected by variations in pigment concentration in sunscreen lip balm preparations, which can cause an increase or decrease in the SPF value and impact on differences in the product's ability to absorb UV rays. Other factors that can affect the SPF value are differences in emulsions and interactions of carrier components, such as esters, emollients, and emulsifiers contained in lip balm preparations. In addition, excipients (candelilla wax and beeswax) and other active ingredients can also produce UV absorption bands so that sunscreens are able to block UV rays, especially UV B.

Table 6. Results of SPF value measurements

Formulations	SPF value	Protection category
F0	38.7793 $\pm$ 0.1427	Ultra-protection
F1	47.9333 $\pm$ 0.0331	Ultra-protection
F2	49.6813 $\pm$ 0.0181	Ultra-protection
F3	48.8302 $\pm$ 0.0525	Ultra-protection
F4	50.4806 $\pm$ 0.0581	Ultra-protection
F5	48.0885 $\pm$ 0.0059	Ultra-protection
F6	51.1674 $\pm$ 0.0364	Ultra-protection

4. Conclusion

Cincalok offers bioactive compounds that have the potential to be used in cosmetic preparations. In this study, the extraction of the bioactive compound astaxanthin from cincalok using VCO has been carried out to produce VCOA, which is directly applied as a lip balm ingredient. Variations in VCOA concentration affect the physical properties of lip balm, such as homogeneity, pH, melting point, color, and SPF value. In this study, up to the use of VCOA at the highest concentration, it produced lip balm with good homogeneity, pH, and melting point in accordance with SNI, the most striking color, and the highest SPF value. The results of this study are expected to be the basis for further research such as moisture testing and antioxidant activity testing of lip balm.

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