



Antioxidant Properties of Coconut-Sugar Instant Coffee Beverages Produced using Red Palm Oil and Coffee Powder

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Abstract

Free radicals increase in oxidative stress that cause the degenerative diseases. Fortunately, dietary antioxidants reduce oxidative stress. Dietary sources of antioxidants include coffee (a popular drink among people) and red palm oil (RPO, a compound that high in antioxidant such as beta carotene and tocopherol). This study examined the nutritional profile of instant coffee beverages (ICB) formulated with different RPO and coffee powder concentrations. RPO was added at 0.3%, 0.4%, and 0.5%. The coffee powder was added at 6%, 8%, and 10%. The beverages were analysed three times. Outcomes were a percentage of DPPH inhibition, tocopherol content, total phenolic content, beta-carotene, also water content. Analysis of variance were used in analysis of data obtained and Duncan multiple range tests was followed if there were significantly different. Higher coffee concentrations corresponded to higher total phenolics and DPPH inhibition percentages but lower tocopherol and beta-carotene. Increased RPO increased beta-carotene and tocopherol content. The coffee drink with an RPO of 0.4% and coffee powder of 8% had the best chemical profile. This antioxidant-rich ICB had the following characteristics: moisture of 5.33%, beta carotene of 704.83µg/100g, total phenol of 2.88%, DPPH of 77.55%, and tocopherol of 0.71%. An ICB enriched with RPO could become a functional health drink and antioxidant source.



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Introduction

Free radicals can increase oxidative stress, a factor in the development of degenerative diseases like diabetes and hypertension.¹ To reduce oxidative damage, people can consume dietary antioxidants such as red palm oil (RPO). The amount of carotenoid pigment in RPO is high at 600 to 1000 ppm.² RPO has 500–700 ppm of carotenes³ like beta-carotene (pro-vitamin A). RPO also contains vitamin E (560–1000 ppm). "Vitamin E" refers to four groups of tocopherols (α -, β -, γ -, and δ -tocopherols) and also four group of tocotrienols (α -, β -, γ -, and δ -tocotrienols). Vitamin E has antioxidant properties, increases hepatocellular function, and lowers plasma glucose levels.² In RPO, the forms of Vitamin E include tocopherol (18–22%), tocotrienol (78–82%), and lycopene (18.5–38 ppm).⁴ The most potent fat-soluble antioxidant is alpha-tocopherol.² Tocopherol functions as an antioxidant and a free radical scavenger, and α -tocopherol breaks radical chains.⁵ Although tocotrienols have less oral bioavailability than tocopherols, they have superior antioxidant action.⁶ Compare to tocopherol, the antioxidant properties of tocotrienol is more effective. Tocotrienol have unsaturated side chain, which can penetrate molecules more deeply and facilitate better penetration. The tocotrienol in RPO reduced oxidative stress, dyslipidemia, and blood glucose in diabetic rats.⁷

To increase RPO consumption, RPO can be added to a widely consumed product like coconut sugar. Coconut sugar is formed by evaporating coconut sap.⁸ When 0.3% RPO was added during the latter stages of processing, coconut sugar solidified at 118°C. RPO did not change the product's sensory qualities (acceptance of taste and texture as well as overall acceptance). The final product contained between 1337 μ g and 3946 μ g of β -carotene per 100g. Intervention of the product for two weeks on rats can increase the reserves of liver retinol (as a vitamin A status indicators) beyond the cut-off point value. Immunoglobulin G (a measure of heightened immunological function) increased nearly threefold.⁹ Additionally, feeding coconut sugar supplemented with RPO can improve the weight gain of vitamin A-depleted rats.¹⁰

Coffee beans contain polyphenols, alkaloids, tannins, and saponins.²¹ In addition, they contain

minor amounts of lignans and anthocyanins.²³ Robusta coffee beans contain chlorogenic acid, which has a concentration of 9.0%.²³ The two polyphenols found in coffee in the highest concentrations are chlorogenic acid and caffeic acid; 90% of all the phenol in coffee is made up of chlorogenic acid.²² Chlorogenic acids (CGAs) contain antioxidant, anti-inflammatory, antidiabetic, anti-obesity, hepatoprotective, antibacterial, and anti-hypertensive activities.²⁶ In addition to chlorogenic acid (8.7 – 11.7%), coffee beans contain antioxidant compounds such as phenolic compound of 36.26 up to 351.35 mg GAE/g, flavonoids of 13.74 up to 104.05 mg QE/g, FRAP of 30.30 up to 175.89 mg GAE/g, ABTS of 14.45 up to 42.65%, and DPPH percentage inhibition of 92.23 up to 95.39%.^{15,16} These substances have health benefits; drinking green and light coffee extracts helps to slow the growth of prostate cancer tumours.¹⁷ Thus, the creation of coffee beverages high in antioxidants could help meet antioxidant demands, particularly among smokers.

An antioxidant-rich instant coffee beverage made with coconut sugar and RPO (ICB) could reduce the oxidative stress from cigarette smoke and prevent degenerative diseases. The nutrient-rich RPO would also increase the competitive value and use value of coconut sugar. This study examined the effect of different concentrations of RPO and coffee powder on the antioxidant properties of an ICB.

Materials and Methods

Study Design

This experimental study was conducted at Food Technology Laboratory at University of Jenderal Soedirman in Purwokerto, Indonesia. A completely randomised design was used. The ICB was made with coconut sugar and different concentrations of RPO and coffee. The first factor was the RPO addition at levels of 0.3%, 0.4%, and 0.5%. The second factors were coffee powder addition of 6%, 8%, and 10%. The ICBs were created and tested three times.

The parameters observed were: DPPH inhibition percentage using spectroscopy, total tocopherol using HPLC,¹¹ total phenol using spectrophotometry,¹² beta-carotene using HPLC at 450 nm wavelength,¹³ and water content using the gravimetric method.¹³

Sample Preparation

The coffee was obtained from the powdered coffee industry in Purwokerto, Central Java, Indonesia. It was sifted using a sieve with a mesh size of 80.

The ICB was made by modifying the process of producing coconut sugar from coconut sap by replacing vegetable oil with RPO:

- Purification of coconut sap: Coconut sap was acquired by "tapping" palm flowers. The coconut sap was cleansed of contaminants using a staged process that included 60 mesh filtrations, boiling, and cooling. The precipitate was obtained by decantation. In a final purification step, re-filtration was performed with a 200-mesh filter.
- Cooking the coconut sap: After measuring the pH and Brix level of clean coconut sap, the sap was cooked. RPO was added (0.3%, 0.4%, 0.5% in accordance with the planned treatments) once the temperature reached 102°C. Next, the coffee powder was added in accordance with the predetermined treatments (6%, 8%, 10%).

The amounts of RPO and coffee powder were corrected by the Brix degrees of the sap at 20°Brix. The equation was:

Amount of RPO/coffee addition = Brix degrees of raw sap / 20 x amount of RPO/coffee treatment x volume of sap

The mixture was stirred continuously. Heating was stopped when the cooking temperature reached 119°C. The solidification stages followed. The stirring was done continuously until a semi-solid mass formed. Finally, the substance was ground and sifted through a 16-mesh screen to create coffee granules.

Tocopherols Assays

Tocopherol extraction was performed with minor modifications to the procedures of Pertuzatti *et al.*¹¹ Cold acetone were used to obtain the ICBs' extracts and then mixed with petroleum ether. Then followed with centrifuged at 9000 rpm for 10 min after the ethereal phase was separated and after that transferred to a vial of 1.5mL for analysis of tocopherol.

A high-performance liquid chromatography system (SHIMADZU, Kyoto, Japan) was used to perform the tocopherols identification and quantification. The system consisting of an LC-10ATVP delivery pump of solvent, column oven, degasser of FCV-10ALVPc, the rheodyne pump of DGU-14A, the SCL-10AVP, CTO-10ASVP, and a SIL-10AF autosampler. The analytical reversed phase column was used in performed chromatographic separation, Shim-Pak CLC-ODS (4.6 mm x 150 mm x 4 mm), and the stationary phase used was the octadecyl groups. Used a wavelength of emission of 330nm and wavelength of excitation of 290nm of a fluorescence detector RF e 10AXL. Using the initial mobile phase of isopropanol/acetonitrile/methanol (10:50:40, v/v/v) for 10 minutes, a total of 10 mL of sample was injected. As linearly, a mobile phase was altered to isopropanol/acetonitrile/methanol (5:30:65, v/v/v) up passed to 12 minutes. Next, it was returned linearly to the initial mobile phase up to 15 minutes. A 1 mL per minute of constant flow was used. To identify the peak was by comparing it to the tocopherol standards. The results were expressed as g tocopherol per 100 g of coffee granule (%).

Total Phenolics Assays

Folin-Ciocalteu assay reported by Hammerschmidt & Pratt was used for determining the total phenolics.¹² A 0.5 mL sample of the extract was mixed with 0.5 mL of the reagent of Folin-Ciocalteu. After that, 1 mL of 7.5% Na₂HCO₃ solution was added to the mixture, and adjusted the volume up to 10 mL with distilled water. The mixture was vortexed for 30s and kept in a dark place for 30min. The absorbance was measured at 750nm using a UV-Vis spectrophotometer (UV-1800, Shimadzu Corporation, Japan). Total phenolics (%) was calculated with the equation:

Total phenolics = (X x V x Fp)/(total sample (g)) x 100%

X = total phenolics of the standard curve

V = extract volume

Fp = dilution factor

Determination of 1,1-Diphenyl-2-Picrylhydrazyl (Dpph) Radical Scavenging Activity

DPPH (1,1-diphenyl-2-picrylhydrazyl) is a useful free radical for determining the activity of antioxidant of

constituents in an extract. It reacts with a substance and contributes an atom of hydrogen. By taking one H atom from an antioxidant or an electron donor to generate DPPH, the DPPH radical is stabilised. The ascorbic acid equivalent is used to represent DPPH radical capture activity. Antioxidants can diminish the DPPH radical, which changes the colour of stabilised DPPH to yellow. This technique can demonstrate how the coffee beverages' active ingredients trap DPPH radicals.²⁴

Estimation the donation capacity of electron or hydrogen atom of ICBs was from bleaching of a purple-coloured DPPH radical methanol solution. An aliquot of the extract or GSH (400 μ L) (1 mg/mL) was mixed with 200 μ L of distilled water. After that, a total amount of 600 μ L of the DPPH solution in methanol of a 0.4mM was added and left in the dark for 30 min. Using a Healicom 721S (China) UV-visible spectrophotometer at 516 nm, the absorbance was taken.

The percentage inhibition of DPPH was calculated using the following equation:

$$\text{DPPH inhibition(\%)} = \frac{(\text{Abs control} - \text{Abs sample})}{(\text{Abs control})} \times 100 \%$$

Beta Carotene Assays

Beta carotene content in the ICBs' coffee granules was analysed according to AOAC methods.¹³ A 1g sample of the coffee granules was extracted with hexane and acetone (1:1), then filtered using a Buchner funnel under vacuum. The resulting filtrate was put into a test tube to be dried with N₂. The dried filtrate was added with 4 mL of 5% KOH and methanol, then shaken and aerated for 30 minutes.

The extract was heated in a water bath at 60°C for 30 minutes. Next, 4 mL of deionised water and 8 mL of hexane were added. These steps were repeated with the addition of 4 mL of deionised water and 6 mL of hexane; the sample was then shaken for 1 minute, and the top layer of the extract was taken and collected. The filtrate obtained was centrifuged at 2000 rpm for 5 minutes.

The organic phase was removed and dried over N₂. The dry residue was combined with 5 mL of 5% CHCl₃ in methanol, then dried and aerated for 30 minutes. The extract was then allowed to stand in a

cooler at -20°C for 12 hours and then dried with N₂. The dry residue was added with 2 mL of acetonitrile (ACN): dichloromethane: methanol (65:30:5) as the mobile phase.

The β -carotene standard was mixed in petroleum ether, evaporated, and mixed with dichloromethane. Standard concentrations were demonstrated spectrophotometrically using the Etem molar extension coefficient of 1% = 2530. Different concentrations were used for the HPLC analysis and plotting of the standard graphs. The correlation coefficient was calculated to estimate the linearity between the standard concentration and the peak area/graph. Samples were diluted for injection and separation. The analysis was related to the average solvent flow at 1.5 mL per minute with a detector sensitivity (AUES) of 0.02 and a standard wave of 450 nm using the formula:

$$\text{Beta carotene (ppm)} = \frac{(\text{sample peak area})}{(\text{sample peak standard})} \times \text{standard concentration} \times \text{dilution factors}$$

Statistical Analysis

Data were analysed by ANOVA at the 95% confidence interval. If there were significant differences, the analysis was continued with Duncan's multiple range test.

Results

Water Content

The amounts of RPO and coffee both had a substantial impact on the water content of the ICBs. Water content increased as the amount of coffee increased. In contrast, water content decreased as RPO increased (Figure 1). The average water content of the coffee mix was 4.75% (for a coffee addition of 6%), 5.05% (for 8%), and 6.05% (for 10%). The mean water content of the coffee mix was 5.87% (for RPO of 0.3%), 5.23% (0.4% RPO) and 4.81% (0.5% RPO).

Total Phenolics Content

The quantity of RPO or coffee added to the ICBs had no discernible impact on total phenolics (Figure 2). Nonetheless, the average phenolics content increased in proportion to the amount of coffee added. In coffee additions of 6%, 8%, and 10%, the mean values of total phenolics were 2.38%, 2.60%, and 2.68%, respectively.

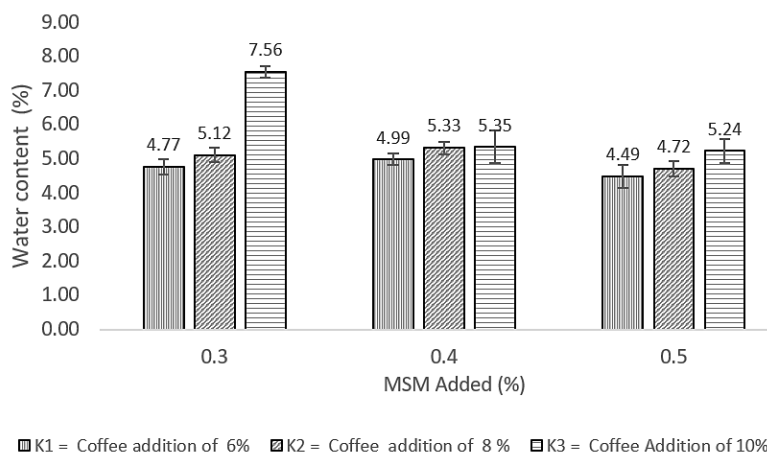


Fig.1: The impact of coffee and red palm oil addition on the water content of the coconut sugar-based instant coffee drink

Table 1: The results of Parameters quantitative analysis of antioxidants properties

Total Phenolics	Tocopherol	DPPH% inhibition	beta carotene
M1K1 2.20 ± 0.325	0.56 ± 0.030	70.8±0.955	575.3±5.386
M1K2 2.44 ± 0.251	0.55 ± 0.0179	75.8±1.389	460.8±16.916
M1K3 2.44 ± 0.185	0.47 ± 0.0084	76.4±1.141	478.7±55.053
M2K1 2.49 ± 0.327	0.72 ± 0.0053	73.3±5.85	969.7±41.96
M2K2 2.88 ± 0.461	0.71 ± 0.0137	77.6±0.864	704.1±34.029
M2K3 2.91 ± 0.195	0.56 ± 0.0314	78.4±1.644	569.5±28.801
M3K1 2.47 ± 0.155	0.85 ± 0.0622	78.6±2.664	1256.8±67.795
M3K2 2.49 ± 0.335	0.84 ± 0.0054	79.5±0.407	997.3±49.367
M3K3 2.70 ± 0.308	0.77 ± 0.0394	82.1±1.630	750.6±34.271

Results are expressed as the mean ± standard error.

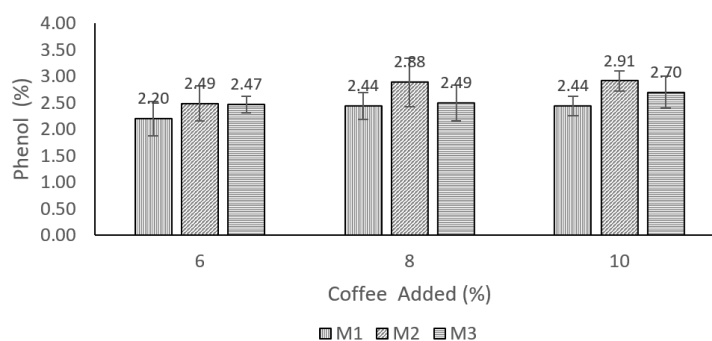


Fig. 2: Effect of red palm oil and coffee addition levels on total phenolics content of a coconut sugar-based coffee drink

Antioxidant Activity (DPPH Inhibition Percentage)
 Figure 3 illustrates how the ICB can scavenge DPPH radicals. The antioxidant activity of the ICBs

was significantly affected by the addition of coffee powder and RPO. The antioxidant activity increased with the addition of coffee or RPO. The mean DPPH

inhibition percentages after adding 0.3%, 0.4%, and 0.5% of RPO were 74.34%, 77.62%, and 80.05%, respectively. The mean DPPH inhibition percentages

after adding 6, 8, and 10 % of coffee were 74.25%, 77.62%, and 78.95%, respectively (Figure 3).

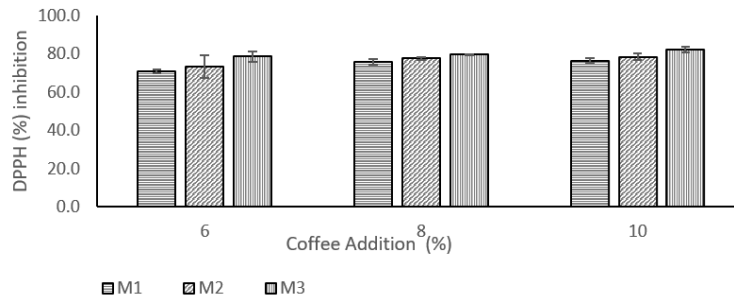


Fig. 3: Effect of red palm oil and coffee addition levels on DPPH inhibition percentage of instant coffee drink

Tocopherol Content

The amount of RPO or coffee considerably affected the total tocopherol content of the ICBs. Mean tocopherol content ranged from 0.47% to 0.85%. Total tocopherol content increased as more RPO was added. On the other hand, tocopherol content decreased as more coffee was added (Fig 4). After

the addition of 0.3%, 0.4%, and 0.5% RPO, the total tocopherol content (vitamin E) of the ICB was 0.526%, 0.664%, and 0.819, respectively. However, the total tocopherol after the addition of 6%, 8%, and 10% of coffee was 0.708%, 0.702%, and 0.599%. The amount of tocopherol increased as RPO increased or as coffee decreased.

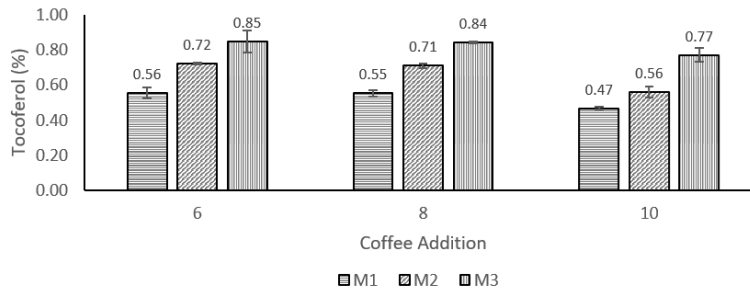


Fig. 4: Effect of red palm oil and coffee addition levels on total tocopherol of coconut-sugar-based coffee drink

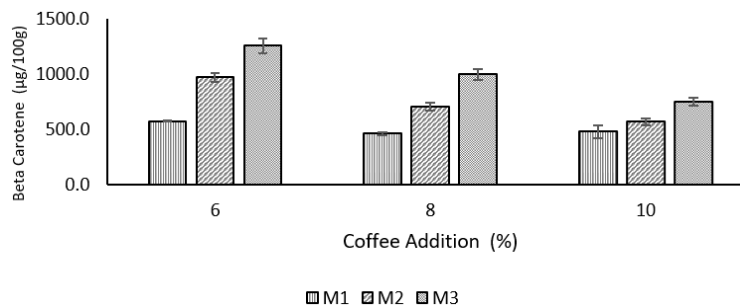


Fig. 5: Effect of adding coffee and red palm oil on the beta-carotene content of coconut-sugar-based instant coffee drinks

Beta-carotene Content

The amount of both RPO and coffee and their interaction had a significant effect on the amount of beta-carotene in the ICBs. The amount of beta-carotene in the product increased as more RPO was added. On the other hand, beta-carotene decreased as more coffee was added (Fig 5). Average beta-carotene in the ICBs treated with 0.3%, 0.4%, and 0.5% RPO was 504.93 µg/100 g, 747.79 µg/100 g, and 1001.17 µg/100 g, respectively. The average beta-carotene after adding 6%, 8%, and 10% coffee was 933.93 µg/100 g, 720.74 µg/100 g, and 599.62 µg/100 g, respectively.

There was a decrease in the number of components (including the beta-carotene content) present per unit weight with the rise in the amount of coffee added. This decrease appeared to be related to the enhanced yield that was consistent with the increase in coffee added during the process.

Discussion

Most smokers are also coffee drinkers. In addition to chlorogenic acid (8.7 – 11.7%), coffee beans contain antioxidant compounds such as phenolic compound (36.26 up to 351.35 mg GAE/g), ABTS (14.45 up to 42.65%), flavonoids (13.74 up to 104.05 mg QE/g), and DPPH (92.23 up to 95.39%), FRAP (30.30 up to 175.89 mg GAE/g).^{15,16} Drinking green and light coffee extracts helps to slow the growth of prostate cancer tumours.¹⁷ Thus, the creation of coffee beverages high in antioxidants could help meet antioxidant demands, particularly among smokers.

When more coffee powder was added in this study, the ICBs' moisture content increased. The method for generating ICBs involved vaporizing coconut sap and adding different concentrations of RPO and coffee. Coffee has a fibre that is hydrophilic (or easily binds water).¹⁸ Coffee contains crude fibre of 8.25–10.64.¹⁶ The presence of coffee would make some water in the coconut sap bind the fibre, inhibiting the evaporation process of coconut sap. As a result, the finished product would include more water when more coffee was added.

On the other hand, the ICBs' moisture content decreased as RPO increased. The presence of oil in a solution system accelerates the evaporation of the

water and enhances the amount of heat received by the system. Oil dissipates heat more effectively than water because it heats up more quickly and has a lower heat type. In this study, RPO replaced oil. RPO aids heat transfer into the material; thus, with more RPO, water release was faster. Although oil heats up more quickly than water and has a lower heat type, oil has a better heat dissipation mechanism.²⁰ Thus, the rate of water evaporation increased with the addition of more RPO.¹⁹

This research revealed that the amount of RPO or coffee had no discernible impact on the total phenol content of the ICBs. Nonetheless, phenol content on average overall was higher (not significant) with increased coffee. On the other hand, the addition of RPO or coffee boosted antioxidant activity. The total tocopherol content was significantly influenced by the amount of RPO or coffee added. RPO increased vitamin E. These findings are consistent with the high nutrient content of both coffee and RPO as described earlier.^{2, 4, 5, 21-24}

Beta-carotene levels in the ICBs increased with the addition of RPO and fell with the addition of coffee. The RPO utilised in this study had a beta-carotene content of 806.210 ppm, according to the analysis. As a result, adding more RPO raised the product's beta-carotene content.

DPPH (1,1-diphenyl-2-picrylhydrazyl) is a free radical that is useful for determining the antioxidant activity of constituents in an extract. It reacts with a substance and contributes an atom of hydrogen in the process. By taking one H atom from an antioxidant or an electron donor to generate DPPH, the DPPH radical is stabilised. The ascorbic acid equivalent is used to represent DPPH radical capture activity. Antioxidants can diminish the DPPH radical, which changes the colour of stabilised DPPH to yellow.

Conclusion

The final ICB produced in this investigation had a water content of 5.33%, beta carotene of 704.83 g/100 g, total phenol of 2.88%, antioxidant activity (DPPH inhibition percentage) of 77.55%, and tocopherols (vitamin E) of 0.71%. The addition of 0.4% RPO and 8% coffee powder produced the

best chemical characteristics. People who frequently encounter free radicals, including smokers, may benefit from an ICB fortified with RPO antioxidants and coffee powder.

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Conflict of Interest

The authors do not have any conflict of interest.

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