A Study on the Characterization and Color Stabilities of Harmless Food Dye Extracted from Kya-ni (*Nymphaea rubra* Roxb. Ex Andrews)

Nwe Nwe Aung[1], Min Thu Htet[2], Ni Ni Than[3], Cho Cho[4]

Abstract

Food colorings are of two types: artificial and natural. Natural colors are healthier than synthetic colors. Natural colors are pigments derived from various fruits, flowers, plants, and animal sources, making them more environmentally friendly than synthetic colors. For that reason, this study was conducted to produce an easy-to-extract food dye that is inexpensive. Kya-ni, known as the red water lily, is a plant that grows easily in ponds. In this study, the petals of the Kya-ni flower *(Nymphaea rubra* Roxb. ex Andrews) were used to make natural food dyes. The dried petals of Kya-ni were investigated by phytochemical tests. It revealed the presence of α-amino acids, carbohydrates, flavonoids, glycosides, phenolic compounds, reducing sugars, saponins, tannins, and steroids, whereas alkaloids, cyanogenic glycosides, and starch were absent in this sample. The nutritional values of the dried petals of Kya-ni were determined by the AOAC method. Heavy metal constituents of the dried petals of Kya-ni were determined by Atomic Absorption Spectroscopy (AAS). The food dye samples were extracted from dried petals of Kya-ni by using water and acetic acid. The obtained dye samples were denoted as K-W for water extraction and K-A for acetic acid extraction. These dyes were characterized by UV and FT IR spectroscopic methods. The colour stability of these food dyes changing from red to yellow was examined on days 1, 7, and 14 by a Lovibond Tintometer. It was found that the colour stability of K-W and K-A was more stable in red colour than in yellow colour. From the overall results, the two colors of natural food dyes, K-W (brown) and K-A (red), are good natural dyes for food products. According to the characteristics and color stability results, Kya-ni food dyes are safe, healthy, and environmentally friendly products, and they can be applied as coloring agents for food processing.

Keywords: Kya-ni, petals, natural food dye, color stability, coloring agent

Introduction

Some pigments naturally occurring in plants are used in food coloring. For centuries, people in Southeast Asia have been using natural dyes to color food, especially for traditional meals. This practice is still widespread in Southeast Asia, although the use of many plant pigments is quite limited. It is common practice to extract pigments directly from fresh materials. Besides the color, people often also appreciate the typical flavor and taste given of the food. People are often attracted by the color first, then the flavors, the structure, and finally, the nutritional value. Synthetic dyes are not always harmless to humans. To ensure the safety and health of consumers, most countries have popularly gazetted regulations on food colorings.

However, a food coloring permitted to be applied in one country may be prohibited in other countries. In general, using natural dyes is safer than using synthetic dyes (Sander Myint, 2013; Nwe Nwe Aung *et al*., 2020).

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[1] Dr, Associate Professor, Department of Chemistry, University of Distance Education
[2] Mg, MSc. Candidate, Department of Chemistry, University of Yangon
[3] Dr, Professor & Head, Department of Chemistry, University of Yangon
[4] Dr, Pro-Rector, University of Yangon

Advantages of the Use of Natural Food Dyes

The benefits of natural food dyes are as follows:

The production of natural dyes using raw materials is plentiful.

Any harm to human skin is not caused by natural dyes. No hazards are anticipated in their production, but some of the synthetic dyes can cause health effects.

The manufacture of natural dyes cannot cause not only chemical reactions but also pollution (Freund, 1988).

Disadvantages of the Use of Natural Food Dyes

Several potential benefits are biocolorants. Their collection extraction is tedious and has a low color value. The synthetic dyes cost less than natural dyes. Synthetic dyes are unstable and difficult to reproduce because this agro-product is reported in terms of season, place, species, maturity period, etc. Natural dyeing has not yet been completely explored. Problems in the food industry arise during processing because of sensitivity to temperature, oxygen, light, and pH. Natural dyes are decolorized or degraded during storage. Those disadvantages hinder their popularity (Gokhale and Tatiya, 2004).

***Nymphaea rubra* Roxb. ex Andrews (Kya-ni)**

Nymphaea rubra Roxb. ex Andrews, locally called Kya-ni and belonging to the family Nymphaeceae, is a wild aquatic plant with erect perennial rhizomes or rootstocks that anchor it to the mud at the bottom. The rhizomes produce slender stolons. This plant is commonly known as the 'water lily'. The most important parts of *Nymphaea,* like the rhizomes, young leaves, and peduncles, are used for food and vegetables, and then the flowers are used for ornamental purposes. Water lilies are such beautiful and showy, sweet-scented flowers that several members of the Nymphaeceae are of great ornamental value for aquatic gardening (Sharma, 2001). The red water lily is found in shallow lakes and ponds mainly in temperate and tropical Asia. In many aromatherapy centers, the vibrant colors and sweet fragrances of Nymphaea flowers have been used as therapeutic agents, and the extraction of the flowers is also applied in many cosmetic products (Jirapong *et al.*, 2012). It is the national flower of Myanmar. In local cultures, the flower is mainly used in religious ceremonies, such as worshipping Buddha, and also in the preparation of different medications by herbal practitioners.

Botanical Aspect of *Nymphaea rubra* Roxb. ex Andrews (Kya-ni)

Scientific Classification

Family	:	Nymphaeaceae
Botanical Name	:	*Nymphaea rubra* Roxb. ex Andrews
English Name	:	Red Water Lily
Myanmar Name	:	Kya-ni
Part Used	:	Petals

Figure 1.The plants of *Nymphaea rubra* Roxb. ex Andrews (Kya-ni)

Description

The red water lily, an aquatic plant, has erect perennial rhizomes that attach to the bottom of the mud. Slender stolons are produced by rhizomes. Its leaf blades can be found above the water. The heart-shaped water lily can be below 15 to 26 cm, papery, and abaxially densely pubescent.

Water Purification Ability

Although there is limited research on flower color variations and formation mechanisms, the water lily has a background of blue flowers and displays an exceptionally wide diversity of flower colors, from purple to red to blue to yellow, that is found in nature. Like lotus, water lilies can be used as an ornamental plant as well as an important water purification plant. The roots of the water lily may absorb poisonous substances like heavy metals such as mercury, lead, and phenol. They can filter the microorganisms in the water. Therefore, it includes an important part for water purification applications (Li *et al*., 2005; Shi *et al*., 2009).

Materials and Methods

Sampling of Flowers

The flowers of *Nymphaea rubra* Roxb. ex Andrews, the red water lily (Kya-ni), was collected from Hlaing Campus at Yangon University in December 2020. These samples were identified by authorized botanists at the Botany Department, University of Yangon. Samples were cleaned with water to remove impurities, and then the samples were air-dried at room temperature for one week. These selected samples were kept in an airtight container to prevent moisture changes and contamination.

Preliminary Phytochemical Investigation on the Petals of Kya-ni

The phytochemical investigation of plants is a major and important experiment due to the classification of groups of chemical substances present in them (Marini-Bettolo *et al.*,1981).

Determination of Some Nutritional Values of the Raw Sample, Kya-ni

The investigation of the percentages of moisture content, ash, fats, fibers, proteins, and carbohydrates in the raw powder sample of Kya-ni was done by the A.O.A.C. A.O.A.C. 2000 methods. The results are illustrated in Table 1 and Figure 2.

Determination of Trace Elements by Atomic Absorption Spectrophotometry

5.0 g of the sample was ashed in a pre-weighed porcelain crucible by first smoking off the fat without burning. The crucible was kept in the furnace at 550 °C overnight until a white ash of constant weight was achieved. Accurately, about 0.1 g of ash was weighed and treated with 2 mL of concentrated hydrochloric acid. The resulting solution of the ash sample was evaporated to dryness, dissolved in 6 cm^3 of 25 % HCl solution (volume by volume), and then centrifuged. The centrifuged solution was decanted, and the clear solution was made up to 100 cm^3 with deionized water. The resultant solution (10 mL) was pipetted accurately and made up to 100 mL with deionized water again. The sample solution prepared was now ready for the analysis of trace elements by AAS. The resultant data are described in Table 2.

Extraction of K-W and K-A from Kya-ni and Their Yield Percent

The sample (10 g) and 125 cm^3 of each solvent (distilled water and 5 % acetic acid) were thoroughly added in a conical flask. It was shaken for 45 minutes at 200 rpm (revolution per minute) and allowed to stand for 15 minutes. The mixture was filtered with a clean cloth, and the filtered water was evaporated until a paste was obtained. These paste-form samples were placed in the oven and dried at 110 °C for 2 hours. After complete extraction followed by evaporation to dryness, dye powders K-W (Kya-ni and distill water) and K-A (Kya-ni and 5 % acetic acid) with the respective colors were obtained. The yield percent of K-W and K-A are described in Table 3.

Characterization of K-W and K-A

UV spectroscopy

The UV spectra of K-W and K-A were recorded using a Perkin Elmer. Lambda 25 UV-Visible Spectrophotometer at the Department of Chemistry, University of Yangon. The resultant spectra are presented in Figures 3, 4, and Table 4.

FT IR spectroscopy

The infrared spectra of K-W and K-A were recorded using a Perkin Elmer Spectrum Two (UATR Mode) Fourier Transform Infrared Spectrophotometer at the Department of Chemistry, University of Yangon. The spectra are shown in Figures 5 and 6.

Determination of Color Stability of K-W and K-A at Various Storage Times

2 % (w/v) sample solution was carefully added into 1 and ¼ inch glass cell and loaded in the Lovibond Tintometer, Model E, England. The illumination of the room was loaded but not sufficient to give total darkness, the names of two color slides among the available color slides (red and yellow) were pulled and the color of the sample solution was matched with them. The shade control was altered such that a full match was obtained. The color measurement was done at the Small Scale Industries

Department, North Okkalapa Township, Yangon Region (Khine Zar Thwe, 2015). The results are shown in Tables 5 and 6. The color stability of K-W and K-A at various storage times are shown in Figures 7 and 8.

Results and Discussion

Phytochemical Examination of Kya-ni by the Test Tube Method

A preliminary phytochemical screening was performed in order to identify the different types of compounds present in Kya-ni. A phytochemical test in the Kya-ni indicated the presence of α-amino acids, carbohydrates, flavonoids, glycosides, phenolic compounds, reducing sugars, saponins, tannins, and steroids, whereas alkaloids, cyanogenic glycosides, and starch were absent in this sample.

Determination of Nutritional Values in Kya-ni

According to the nutritional values, moisture of 12.00%, fat of 1.29%, ash of 5.40%, fiber of 22.73%, protein of 2.54%, and carbohydrate of 56.04% were observed in Kya-ni. According to these observations, carbohydrate content was found to have the highest composition of all the nutrients. The results are shown in Table 1 and Figure 2.

Table 1.Results of Nutritional Values in Kya-ni

No.	Parameters	Methods (AOAC)	Contents (%)
1	Water or Moisture	Oven drying method	12.00
2	Ash	Muffle furnace method	5.40
3	Protein	Micro-Kjeldahl's method	2.54
4	Fiber	Manual laboratory techniques	22.73
5	Fat	Soxhlet extraction method	1.29
6	Carbohydrate	[100- (moisture+ fat + ash+ fiber + protein)]	56.04
7	Energy Value (kcal/100g)		245.93

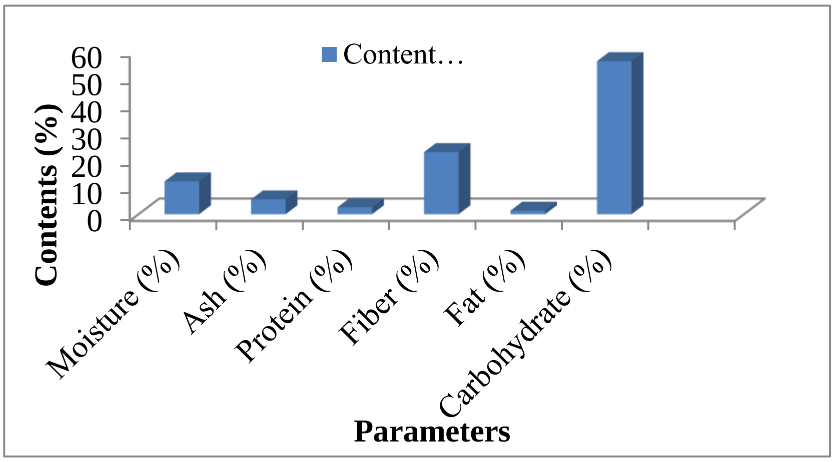


Figure 2 Results of Nutritional Values in Kya-ni

Determination of Heavy Metal Constituents in Kya-ni by AAS

The raw powder of Kya-ni was determined by the AAS method. The contents of lead and arsenic were observed only in trace amounts (0.000001 and 0.000020 ppm, respectively). But Mercury and cadmium were found to be absent in the given sample. The observed amounts of two heavy metals are within the range of WHO standards. The results are presented in Table 2.

Table 2.Heavy Metal Constituents in Kya-ni

Heavy metals	WHO Standard value (ppm)	Observed value (ppm)
Hg	0.001	-
Cd	0.003	-
Pb	0.010	0.000001
As	0.010	0.000020

Extraction of K-W and K-A from Kya-ni and Their Yield Percent

In this research, K-W and K-A were extracted from Kya-ni by using water and 5 % acetic acid. From these results, the yield percent dye powder samples, K-W and K-A (brown color and red color), were found to be 12.00% and 9.60%. Therefore, the yield of K-W was found to be higher than that of K-A. The results are summarized in Table 3.

Table 3. Yield Percent of K-W and K-A

No.	Samples	Solvent	Colour	Yield percent (%)
1	K-W	H_2O	Brown	12.00
2	K-A	5 % Acetic acid	Red	9.60

Characterization of K-W and K-A by the UV-visible Spectroscopic Method

K-W and K-A dye samples were characterized by UV-visible spectrophotometer between 200 and 700 nm. These spectra are shown in Figures 3 and 4.The UV-visible spectra of K-W and K-A are indicated in Table 4. These resultant data confirmed the presence of double bond conjugation in these dyes.

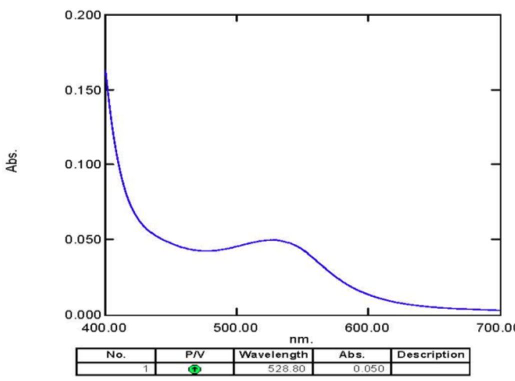


Figure 3 UV-vis spectrum of K-W

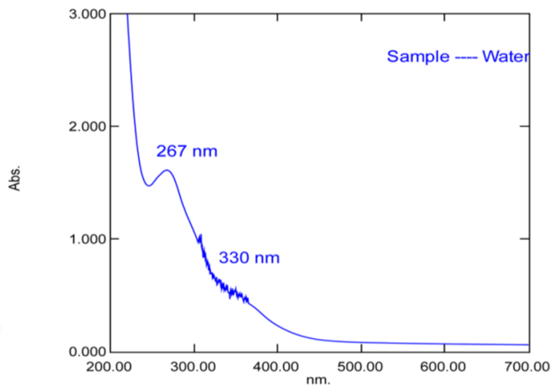


Figure 4 UV-vis spectrum of K-A

Table 4.UV-visible Absorption Wavelengths of K-W and K-A

Extracted Dye	Wavelengths (nm)	Remark
K-W	267, 330	Double bond conjugation
K-A	528	

Characterization of K-W and K-A by the FT IR Spectroscopic Method

In the FT IR spectra of K-W, the hydroxy group of O-H appeared at 3210 cm^{-1}. In the case of K-A, O-H stretching, or NH, $-NH_2$, NH_4^+, and CN^- groups. It appeared at 3343.88 cm^{-1}. The bending O-H of K-W and K-A appeared at 1327 and 1345.6 cm^{-1} respectively. The absorption band of 1603 cm^{-1} indicated the C=C stretching of aromatic. The absorption bands of 1219.65 and 1093.62 cm^{-1} showed C-N stretching of aromatic N-H, $-NH_2$, NH_4^+, and CN_2 and C-N stretching of $C-NH_2$, C-NH group in the K-A. The absorption band of 1041.63 cm^{-1} showed C-O stretching of C-OH group in K-A. The absorption band of 1196 cm^{-1} of K-W indicated the C-O stretching of the C-OH group. The differences between K-W and K-A can be clearly seen at 3210 and 3343 cm^{-1} .The FT IR spectra of K-W and K-A are shown in Figures 5 and 6.

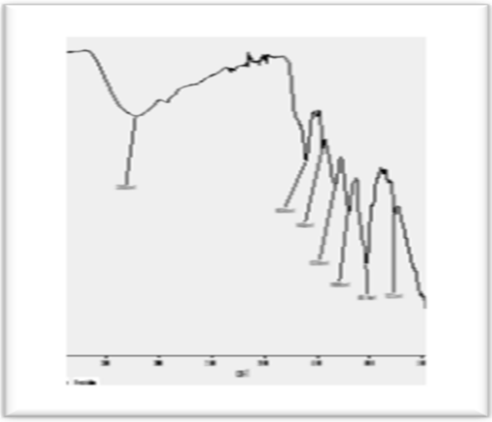


Figure 5.FT IR spectrum of K-W

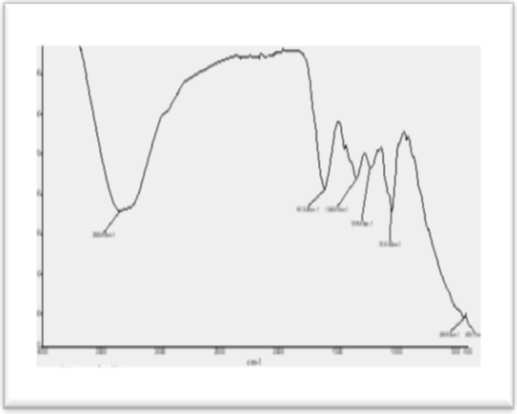


Figure 6. FT IR spectrum of K-A

Color Stability of K-W and K-A at Various Storage Times

Color measurements of K-W and K-A were carried out on days 1, 7 and 14. The changes in color stability of K-W and K-A with respect to storage times are shown in Table 5 and 6. It was found that the stability of red color in K-A increased to 100%, 96.5%, and 84.6%, and that of yellow color in K-W increased to 100%, 50%, and 26% in days 1, 7, and 14, respectively. In K-W and K-A, the red color of the dye was found to be more stable than the yellow color of the dye. The red color dye in K-A is more stable than K-W. But, in the case of yellow, the color stability of K-W was found to be higher than that of K-A. The changes in color stability of K-W and K-A with respect to the storage times are also shown in Figures 7 and 8.

Table 5.Changes of Red Color Stability of K-W and K-A at Different Storage Times

Storage time (day)	Red color stability (%) of dye samples	
	K-W	K-A
1	100 (20)	100 (5.4)
7	65 (13)	96.5 (5.2)
14	43 (8.6)	84.6 (4.6)

Table 6.Changes in Yellow Color Stability of K-W and K-A at Various Storage Times

Storage time (day)	Yellow color stability (%) of prepared Dye color	
	K-W	K-A
1	100(15)	100 (3.2)
7	50(7.5)	37(1.2)
14	26(3.9)	20(0.64)

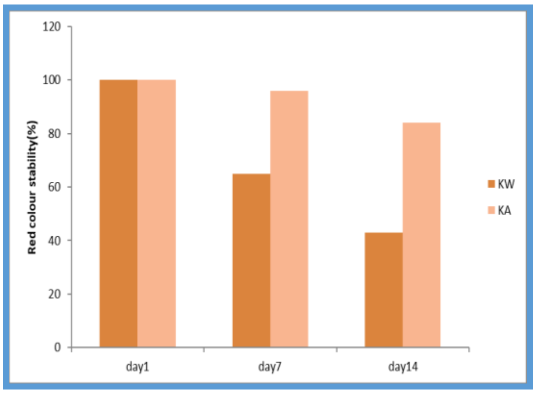


Figure 7.The red color stability of K-W and K-A

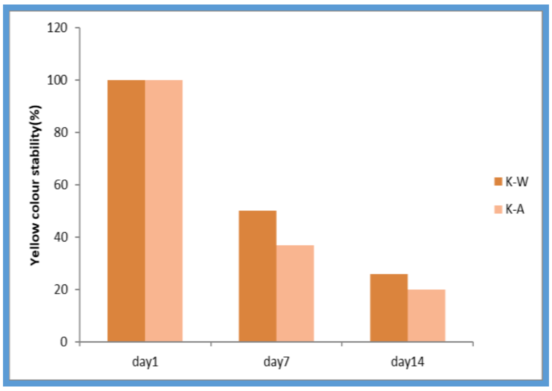


Figure 8.The yellow color stability of K-W and K-A

pH Changes of K-W and K-A at Different Storage Times

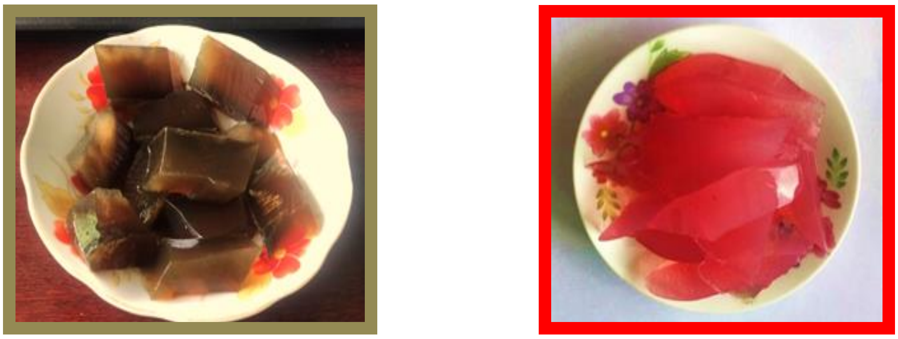
pH was found to increase from 1.02 to 1.26 in K-A at different storage times. But, the pH of K-W was found to have decreased. According to these results, K-A may be more stable than K-W at various storage times. The results are shown in Table 7.

Table 7.pH changes of K-W and K-A at Various Storage Times

Storage time (day)	pH	
	K-W	K-A
1	6.21	1.02
7	5.49	1.14
14	5.42	1.26

Application of K-W and K-A in Food Processing

The food dyes, K-W (brown) and K-A (red) were applied as coloring agents in food processing; agar jelly. Figure 9 (a) and (b) show dyeing agar jelly with K-W and K-A. It was found that the brown and red jellies were attractive to eat and did not change the original taste.



(a) **(b)**

Figure 9. Dyeing agar jelly by using food dye (a) K-W (brown) and (b) K-A (red)

Conclusion

In researching the production of food dye from Kya-ni, the following findings were made:

According to the preliminary phytochemical test, it was observed that amino acids, carbohydrates, flavonoids, glycosides, phenolic compounds, reducing sugars, saponins, tannins, and steroids were present in this sample, whereas alkaloids, cyanogenic glycosides, and starch were absent. Nutritional values of Kya-ni were found to be moisture content (12.00%), ash content (5.40%), fat content (1.29%), protein content (2.54%), and fiber content (22.73%), carbohydrate content (56.04%), and an energy value of 245.93 kcal/100g. According to results from the AAS method, the contents of lead and arsenic in the raw powder of Kya-ni were found to be within the permissible range of the WHO standard. Harmful metals like cadmium and mercury were absent in the sample. Therefore, the selected sample (Kya-ni) can be safely applied as a harmless food dye. The brown and red colors of food dyes (K-W and K-A) were extracted from Kyi-ni by using different solvents (water and acetic acid). The UV-visible spectra of K-W and K-A demonstrated several absorption bands between 200 and 700 nm. These resultant data confirmed the presence of double bond conjugation in these dyes. In the FT IR results, compounds containing the OH group, NH_4^+ group and COO^- group may be present in the K-W. In the case of K-A, -OH or NH, $-NH_2$, NH_4^+ and CN^- groups, it appeared. The color stability of these food dyes changing from red to yellow was examined on days 1, 7, and 14 by a Lovibond Tintometer. It was found that the color stability of K-W and K-A was more stable in red than in yellow. The red color dye in K-A is more stable than K-W. But, in the case of yellow color stability of K-W was found to be higher than that of K-A. pH was found to increase from 1.02 to 1.26 in K-A. But the pH of K-W was found to have decreased. Free of the harmful heavy metals K-W and K-A, brown and red colors were used to make agar jelly as a food dye. It was found that these color-dyed agar jellies were beautiful and attractive without losing their original taste.

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