Effects of Ethanol Treatment on Storage Quality and Antioxidant System of Postharvest Papaya

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Papaya is the fourth most favored tropical fruit in the global market; it has rich nutrition and can be used for medicine and food processing. However, it will soften and mature in a short time after harvest, resulting in a lot of economic losses. In this study, papaya fruits were soaked in 0, 12.5, 25, 50, and 100 ml/L ethanol solutions for 2 h and stored at 25°C for 14 days, by which we explored the effects of ethanol treatment in papaya after harvest. At an optimal concentration of ethanol treatment, color changing of the papaya fruits was delayed for 6 days, and decay incidence and average firmness of the fruits were shown as 20% and 27.7 N, respectively. Moreover, the effect of ethanol treatment on antioxidant systems in the papaya fruits was explored. It was observed that ethanol treatment contributed to diminish the development of malondialdehyde (MDA), ethylene, and superoxide anions. Furthermore, the activities of superoxide dismutase (SOD), catalase (CAT), and ascorbate peroxidase (APX) were promoted than those of control group, while the activities of peroxidase (POD), phenylalanine ammonia-lyase (PAL), and polyphenol oxidase (PPO) were brought down. In addition, the principal component analysis (PCA) showed that PAL, ethylene, and superoxide anions were the main contributors for the maturity and senescence of postharvest papaya. In this experiment, ethanol treatment had the potential of delaying the ripening and maintaining the storage quality of papaya fruits.

Keywords: papaya, ethanol, postharvest physiology, storage quality, antioxidant system

INTRODUCTION

Papaya (Carica papaya L.) is a rapid-growing, latex-producing herbaceous fruit tree and is widely cultivated in the tropics and subtropics (Rogayah et al., 2018). As the fourth most favored tropical fruit in the global market after bananas, mangoes, and pineapples (Xu et al., 2020), the papaya fruit has rich nutrients, carotenoids, and vitamins A and C, as well as minerals such as Mg, K, and Cu (Hardisson et al., 2001; Wall, 2006). In addition to fresh-eating, papaya fruit can also be used for medicine (Karunamoorthi et al., 2001; Wall, 2006). In addition to fresh-eating, papaya fruit can also be used for medicine (Karunamoorthi et al., 2014) and food processing (Mendoza et al., 2008), and contains unique papain for meat tenderization and beer clarification (Liu et al., 2007). Its seed oil can be extracted as a green biofuel (Asokan et al., 2018).
Papaya is a typical respiratory climacteric fruit, which will soften and mature in a short time after harvest. During ripening, the yellowing of the outer pericarp begins at the tip of the stigma, while coloring and softening of the inner flesh develops outward from the endocarp (Paull et al., 1997). During maturation, soluble solids content of papaya towards increase, which varies from 6% to 19% depending on varieties (Sivakumar and Wall, 2013). The ascorbic acidity of “Golden” papaya increases by 20–30% during maturation, while the pericarp pH value and titratable acid content decrease (Bron and Jacomino, 2006). The total carotenoid content of “Pococi” papaya improves from 130 µg/g FW to 5,414–6,214 µg during maturation, and the esterified β-cryptoxanthin content is very high at the early stage of maturation. At the later stage, β-cryptoxanthin ester and (all-E)-lycopene are dominant (Schweiggert et al., 2011). A study has shown that the maturation of the papaya fruit is associated with increased activities of polygalacturonase (PG), pectate lyase (PEL), catalase (CAT), and ascorbate peroxidase (APX) and high level of H2O2 and lipid peroxidation, as well as decreased activities of superoxide dismutase (SOD) and guaiacol peroxidase (GPX) (Pandey et al., 2013).

Ethanol (C2H5OH), a low-toxic plant secondary metabolite, is used as an effective and safe food additive for postharvest preservation and storage of horticultural crops. It was found that ethanol soaking or steam treatment could prolong the life of freshly cut Bougainvillea flowers (Hossain et al., 2007) and inhibit the maturation of melons (Liu et al., 2012) and rottleness of “Red Earth” grapes (Candir et al., 2012). Ethanol vapor treatment repressed chlorophyll catabolic enzyme and related gene expression, which postponed the yellowing of broccoli (Fukasawa et al., 2010). Besides, ethanol treatment could retard the senility of fruits by killing bacterial propagules, fungi, and viruses. Treating bayberry with 1 or 1.5 mL/L ethanol for 2–3 h and 0.5 mL/L ethanol for 3 h could significantly inhibit the germination of pathogenic bacteria and delay the aging of fruits (Wang et al., 2011).

In this study, physiological changes in papaya were studied using different concentrations of ethanol immersion as treatment, aiming to explore the optimal concentration of ethanol treatment for papaya postharvest storage, enrich the theory of papaya postharvest preservation, and provide some reference for production practice.

MATERIALS AND METHODS

Materials and Treatments
Papaya fruits (C. papaya L., cv ‘Sunrise’), harvested in Ledong county, Hainan province, were quickly transported to the laboratory for treatments. A total of 150 papaya fruits with similar size and maturity degree of 70–80% and without mechanical damages, diseases, and insect pests were randomly divided into five groups, soaked in 0.05% sporgon solution for 5 min, and dried naturally. Then, the fruits were soaked in 0, 12.5, 25, 50, and 100 mL/L ethanol solutions for 2 h (which were separately regarded as 0 mL/L ethanol treatment (CK) for the control group and treatment groups A, B, C, and D). After drying the surface of the pericarp, the fruits were transferred to the storage place at 25°C and 80% RH. Three fruits were selected randomly from each treatment group on days 0, 2, 4, 6, 8, 10, 12, and 14 (the day of ethanol treatment was regarded as day 0) for determination of physiological indicators, cryopreserved with liquid nitrogen, and preserved in a refrigerator at −80°C for further analysis.

Measurement of Physiological Indicators

Determination of Decay Incidence and Weight Loss Rate
Ten fruits from each treatment group were selected for observation of decay incidence. When there appeared rottleness, mildew, or black-brown spots on a fruit, it was regarded as decay and recorded.

Weight loss rate was calculated by weighing the fruits on an electronic balance. The formula is as follows:

$$ WLR_n = \frac{w_0 - w_n}{w_n} \times 100\%,$$

where WLRn represents the weight loss rate of the fruit on day N, w0 means the weight on the initiation day, and wn means the weight on day N.

Determination of Firmness, Soluble Solid Content, and Titratable Acidity

Firmness was measured with a sclerometer. Points were chosen from the equatorial surface of each side of every fruit to measure firmness. Soluble solid content was determined with a handheld refractometer (PAL-1; Atago Ltd., Japan). To determine titratable acidity, 5 mL papaya fruit juice was extracted, dissolved in 50 mL distilled water, added with phenolphthalein, and then titrated with 0.1 mol/L, pH 8.3 NaOH solution, and acidity was expressed as the equivalent percentage of malic acid.

Determination of MDA Content

The measuring method of MDA was based on Ding et al. (2015) and slightly modified. Pulp 2 g and 3 mL 0.05 M, pH 7.8 sodium phosphate buffer were homogenized in ice bath and then centrifuged at 4°C and 12,000 g for 15 min. A 2 mL 5 g/L solution of thiobarbituric acid was added into 2 mL supernatant, water bathed for 15 min at 95°C, cooled, and centrifuged for another 15 min. We determined OD450, OD532, and OD600 values and calculated MDA content using the formula:

$$ C = 6.452 \times \frac{OD_{532} - OD_{600}}{0.559 \times OD_{450}}.$$

Determination of Superoxide Anions Content

The determination method for superoxide anions content was referred to Cao et al. (2007). Pulp 2 g and 3 mL 0.05 M, pH 7.8 sodium phosphate buffer were homogenized in ice bath and then centrifuged at 4°C and 12,000 r/min for 20 min. We took 1 mL of the supernatant and added 1 mL of the sodium phosphate buffer and 1 mL of 1 mmol/L hydroxylamine hydrochloride solution. The solution was stirred well and kept at 37°C for 20 min. Then, 1 mL 17 mmol/L p-aminobenzen sulfonic acid solution and 1 mL 7 mmol/L o-naphthylamine solution were added, and the mixture was stirred and kept at 37°C for 20 min. OD530 value was measured, and the blank was adjusted with distilled water.
Determination of Ethylene Content
Ethylene content was determined using an enzyme-linked immunoassay (ELISA) kit (Jiangsu Kete, Jiangsu, China) and referred to Sun et al. (2016). We added papaya pulp samples and standard and antibodies marked with horseradish peroxidase (HRP) to microwells precoated with ethylene antibody and incubated the mixture at 37°C for 60 min. Then, 3,3′,5,5′-tetramethylbenzidine (TMB) was utilized for development of color, which turned blue under the action of HRP and finally became yellow under the activity of acid. Color depth had a positive correlation with ethylene content in the samples. We determined the absorbance at 450 nm and calculated the ethylene content of the samples.

Determination of Enzyme Activities and Total Protein Content
The activities of SOD, peroxidase (POD), CAT, phenylalanine ammonia-lyase (PAL), polyphenol oxidase (PPO), and APX, and total protein content were determined using assay kits (Nanjing Jiancheng, Jiangsu, China) and following the manufacturer’s specification.

Data Processing and Analysis
This study utilized a completely randomized design with a total of three biological repeats. Data were analyzed by one-way analysis of variance (ANOVA). Means were compared using least significant difference (LSD) with a confidence level of $P < 0.05$.

RESULTS AND ANALYSIS

Color-Changing of Postharvest Papaya Treated With Ethanol
It can be observed from Figure 1 that on day 6 of storage, the papaya fruits of the control group began to change color, and that the surface turned yellow in a large area on day 8. For comparison, ethanol treatment had the effect of delaying color transformation, and the effect of treatment on group B (25 mL/L ethanol) was the most obvious, because the color was still green on day 12, followed by treatment group C (50 mL/L ethanol), in which the fruits partially turned yellow. The delayed effect on treatment groups A and D however, was not evident. It indicated that proper concentration of ethanol treatment could delay the color-changing of papaya fruits for 6 days.

Appearance and Flavor Quality of Postharvest Papaya Treated With Ethanol
After 14 days of storage, it can be observed that the decay incidence of treatment groups B and C decreased by 20 and 30% compared with the control group, and that weight loss rate...
FIGURE 2 | (A) Decay incidence, (B) weight loss rate, (C) firmness, (D) soluble solids content, (E) titratable acidity, and (F) total protein content of papaya fruits treated with 0, 12.5, 25, 50, and 100 mL/L ethanol. Values are means ± SD. *Represents significant differences among the treatments (p < 0.05).

decreased by 2.25 and 2.8%, respectively, while average fruit firmness increased by 65.37 and 13.13% (Figures 2A–C). In addition, low concentration of ethanol treatment is beneficial to storage, while higher concentration (treatment group D, 100 mL/L ethanol) was not conducive to storage and may even accelerate decay.

Soluble solid content and titratable acidity could be reduced by ethanol treatment. According to Figure 2D, the peak value of soluble solids content in the control group appeared on day 6 and then declined rapidly, while treatment groups reached peak value on day 8 and after that declined slowly. The titratable acidity of all the experimental groups reached troughs on day 4, but it increased rapidly in the control group and slowly in the treatment group in the following days (Figure 2E).

During storage, protein content generally increased slowly and peaked on day 10 (Figure 2F), which was because of the synthesis of a great number of enzymes in the period of respiratory climacteric. However, ethanol treatment had little effect on total protein content. At the later stage of storage, the total protein content of treatment group B was the highest, although the effect was not obvious.

Contents of Malondialdehyde, Ethylene, and Superoxide Anions
Ethanol treatment could significantly reduce the content of MDA at the later stage of storage, and treatment group B had the best effect, followed by treatment groups C and A (Figure 3A). On day 12, the content of MDA in treatment groups A, B, and C was reduced by 11.78, 22.51, and 17.86%, respectively, compared with the control group (Table 1). In addition, there was no marked quantitative difference in MDA content between treatment group D and the control group on day 14,
indicating that high concentration of ethanol treatment was unsuitable for storage.

**Figure 3B** shows that the ethylene content in the control group increased rapidly on days 6–8, reached the highest point on day 10, and then declined slightly. However, the ethylene content of the treatment groups was significantly lower than that of the control group overall from days 6 to 10. The inhibition effect of treatment groups B and C was the best, in which the content declined by 41.21 and 38.65% on day 8 and 26.53 and 22.79% on day 10, respectively, indicating that ethanol treatment had an obvious effect on repression of the synthesis of ethylene, and that the effect varies with concentration.

Generally, the content of superoxide anions was increasing, and different concentrations had different effects (**Figure 3C**). At the later stage of storage, ethanol treatment can reduce the content of superoxide anions, and treatment group B has the best effect.

**Activities of Oxidases and Antioxidant Enzymes**

**Figure 4A** shows that the peak of SOD activity in the control group appeared on day 8, and that the peak in the experimental group was delayed until day 10. In the whole experiment, the activity of SOD in treatment group B was basically the highest, and the activities of the other treatment groups were also higher than that of the control group, which indicated that ethanol treatment could promote the activity of SOD.

Similarly, POD activity peak was delayed after ethanol treatment (**Figure 4B**). Overall, the POD activity of treatment B remained at a low level, while there was no marked difference between the other treatment groups and the control group, which suggested that suitable ethanol treatment concentration could effectively inhibit POD activity.

**Figure 4C** shows that CAT activity increased on days 2–4 and days 6–8, and decreased rapidly after the peak activity on day 8. In the first 8 days, the activity of treatment group B was the highest and then decreased, with a little difference from that of the other experimental groups, which showed that ethanol treatment mainly affected CAT activity at the first half of storage.

The activity of PAL decreased slightly on days 2–6, reached the highest when respiratory climacteric peaked, and then returned to the previous level (**Figure 4D**). During the latter half of the storage period, PAL activity was significantly reduced by ethanol treatment. Overall, treatment group B had the lowest activity and the best effect of inhibition.

Polyphenol oxidase activity increased rapidly on days 6–10 because of the maturity and senescence of the fruits and respiratory climacteric, while ethanol treatment could reduce the activity of enzymes to some extent compared with the control (**Figure 4E**). During the experiment, the activity of treatment group B was always low, followed by groups A and C, although the difference was not significant, indicating that the effect of ethanol on papaya fruit senescence was not mainly attributed to the inhibition of PPO activity.

**FIGURE 3** | (A) Malondialdehyde content, (B) ethylene content, and (C) superoxide anions content of papaya fruits treated with 0, 12.5, 25, 50, and 100 mL/L ethanol. Values are means ± SD. *Represents significant differences among the treatments (p < 0.05).
Effect of treatment on postharvest papaya after 8, 10, and 12 days of storage.

| Treatment | PAL (µmol/mg prot) | Ethylene (µmol/kg) | SOD (U/mg prot) | APX (U/mg prot) | CAT (U/mg prot) | PPO (U/mg prot) |
|-----------|------------------|-------------------|----------------|----------------|----------------|----------------|
| Control   | 22.44 ± 0.08a    | 0.39 ± 0.06a      | 289.03 ± 1.20a | 29.36 ± 0.65a  | 31.04 ± 0.83a  | 5.49 ± 0.03a   |
| A         | 11.17 ± 0.03a    | 0.12 ± 0.01a      | 12.03 ± 0.15a  | 3.04 ± 0.02a   | 1.23 ± 0.04a   | 0.08 ± 0.01a   |
| B         | 12.47 ± 0.02a    | 0.23 ± 0.01a      | 12.04 ± 0.15a  | 3.04 ± 0.02a   | 1.23 ± 0.04a   | 0.08 ± 0.01a   |
| C         | 12.47 ± 0.02a    | 0.23 ± 0.01a      | 12.04 ± 0.15a  | 3.04 ± 0.02a   | 1.23 ± 0.04a   | 0.08 ± 0.01a   |
| D         | 12.47 ± 0.02a    | 0.23 ± 0.01a      | 12.04 ± 0.15a  | 3.04 ± 0.02a   | 1.23 ± 0.04a   | 0.08 ± 0.01a   |

Values are the mean ± SD, and those followed by same letter are not significantly different at p < 0.05.

Comprehensive Evaluation of Different Treatments Based on TOPSIS

For selecting the suitable concentration of postharvest papaya, we performed the Technique for Order of Preference by Similarity to Ideal Solution (TOPSIS) based on 15 physiological indicators storage day 10 (Table 2). The comprehensive score of treatment B was 0.99, significantly higher than that of the other groups, followed by treatment C (0.56) and control the group (0.53), while that of treatments A and D was lower than CK. It was obvious that treatment B (25 mL/L) was the suitable concentration of ethanol treatment in this study, and we suggested that high or low concentration of ethanol treatment was not conducive to the storage of papaya.

Principal Component Analysis (PCA) of Antioxidant System

A principal component analysis (PCA) was performed based on nine antioxidant indicators of papaya on storage day 10, with which exploring the main factors contribute to storage quality (Table 3). Three principal components were extracted with eigenvalues greater than 1 as standard, and variance contribution rates were as follows: 51.23, 14.74, and 12.52%, and cumulative contribution rate was 78.49%, and the three principal components could represent most of the filtered information. PC1 had the highest contribution rate, and the top three indicators were PAL, ethylene, and superoxide anions; the main influencing factors of PC2 were POD and MDA; PC3 was mainly affected by SOD, MDA, and APX.

The biplot of the PCA is shown in Figure 5. The angles among PAL, ethylene, superoxide anions, MDA, and PPO were less than 90°, indicating that they were positively correlated. Also, they were on the positive half of PC1, which meant that these 5 indicators had a positive effect on PC1 and were not conducive to storage of postharvest papaya.

DISCUSSION

Climacteric fruits mature and age rapidly after harvest, and are accompanied by deterioration, water loss in the flesh, and entire quality decline. Ethanol, a green and safe food additive, could effectively delay fruit ripening and senescence, and improve the overall storage quality, which has been proved in a variety of horticultural crops and is a potent strategy for postharvest preservation. This study demonstrated that the storage quality...
of postharvest papaya was improved, and that the antioxidant system was promoted by ethanol treatment.

**Ethanol Treatment Improved the Appearance Quality of Postharvest Papaya**

In this experiment, ethanol treatment could delay color-changing of papaya fruits for several days, maintain firmness, and reduce fruit decay incidence and weight loss rate, which were beneficial for improving appearance quality. Capotorto et al. (2018) soaked fresh-cut fennel in 0.5% ethanol for 2 min and found that it effectively preserved the visual quality in air at 5°C for 6 days and significantly reduced the blackening of the chopped stem and shell surfaces. The results of Ji et al. (2021) on blueberries indicated that ethanol vapor treatment delayed the decline in firmness and destruction of the cell wall structure of fruits. We presumed that ethanol treatment could also delay

**TABLE 2 | Technique for Order of Preference by Similarity to Ideal Solution (TOPSIS) Analysis of Different Treatments Based on 15 Indicators on Storage Day 10.**

| Treatment | $D^+_i$ | $D^-_i$ | C    | Rank |
|-----------|---------|---------|------|------|
| CK        | 32.15   | 35.65   | 0.53 | 3    |
| A         | 56.35   | 20.85   | 0.27 | 4    |
| B         | 0.84    | 61.13   | 0.99 | 1    |
| C         | 28.75   | 36.00   | 0.56 | 2    |
| D         | 48.40   | 14.11   | 0.23 | 5    |
TABLE 3 | Principal component analysis of the antioxidant system on storage day 10.

|                  | PC1   | PC2   | PC3   |
|------------------|-------|-------|-------|
| MDA              | 0.13  | 0.35  | 0.34  |
| Ethylene         | 0.19  | −0.17 | 0.11  |
| Superoxide Anions| 0.18  | 0.01  | −0.09 |
| SOD              | −0.18 | −0.04 | 0.38  |
| POD              | −0.04 | 0.42  | −0.68 |
| CAT              | −0.09 | −0.55 | −0.29 |
| PPO              | 0.13  | −0.28 | 0.02  |
| PAL              | 0.20  | 0.08  | 0.03  |
| APX              | −0.17 | 0.20  | 0.24  |
| Eigenvalue       | 4.61  | 1.33  | 1.13  |
| Variance contribution rate/% | 51.23 | 14.74 | 12.52 |
| Cumulative contribution rate/% | 51.23 | 65.97 | 78.49 |

**FIGURE 5** | Biplot of principal component analysis of antioxidant system on storage day 10.

**Effect of Ethanol Treatment on Oxidases and Antioxidant Enzymes**

There are effective efficient enzymatic (SOD, CAT, APX, and so on) and non-enzymatic antioxidant defense systems in plants, which protect plant cells from oxidative damage by eliminating reactive oxygen species (Gill and Tuteja, 2010). SOD combined with CAT can transform superoxide free radical (O$_2^-$) into O$_2$ and H$_2$O$_2$; APX participates in the ascorbate-glutathione (AsA-GSH) cycle to remove H$_2$O$_2$, and these enzymes reduce the damage of ROS to the cell membrane (Apel and Hirt, 2004). This study concluded that proper concentration of ethanol treatment could promote the activities of SOD, CAT, and APX, and hasten the antioxidant system, which is finally manifested as delay of fruit ripening and senescence. These were similar to those found on banana treated with ethanol by de França et al. (2019), and further studies of Li et al. (2018) on fresh-cut strawberries found that exogenous ethanol treatment activated the related gene expression of SOD, CAT, and APX, thereby increasing activities of enzymes.

On the contrary, ethanol treatment inhibited the activities of POD, PPO, and PAL, which are the main enzymes inducing enzymatic browning in horticultural crops (Vamos-Vigya and Haard, 1981; Nicolas et al., 1994; Lopez-Serrano and Barcelo, 2002; Jhin and Hwang, 2015). Phenyllalanine could be converted to ammonia and trans-cinnamic acid by PAL, while the latter could be ulteriorly converted to phenylpropanoid compounds, including monophenol and o-diphenol. These phenolic substances could be oxidized by PPO to o-diquinones.
and further oxidized to brown pigment or melanin, thus affecting the appearance and quality of horticultural crops. Exogenous ethanol treatment could restrain the activities of POD, PPO and PAL, reduce browning, and improve fruit quality after harvest, which were the same as the results of Hu et al. (2010) on fresh-cut eggplants and Dong et al. (2016) on fresh-cut burdocks. Similarly, ethanol treatment combined with acetic acid (Huang et al., 2020) and phytoncide (Kim et al., 2014) could notably inhibit the enzymatic browning of lettuce. In addition, PAL is one of the key enzymes regulating anthocyanin biosynthesis (Leyva et al., 1995; Boss et al., 1996), and decline in PAL activity slowed the process of color-changing in papaya.

Major Contributors to Storage Quality and Fruit Ripening Based on PCA

The PCA reflected the contribution of each indicator to storage quality and fruit ripening. In this study, PC1 (51.23%) accounted for half contribution rate, of which three most important indicators were PAL, ethylene, and superoxide anions, followed by PC2 (14.74%) and PC3 (12.52%). It can be seen that the increase in PAL activity and ethylene and superoxide anion content were the main contributors for papaya fruit ripening and senescence after harvest, and that ethanol treatment suppressed PAL, ethylene, and superoxide anions, which delayed the maturation of the fruits.

CONCLUSION

The purpose of this study was to determine the effect of ethanol treatment on postharvest papaya and showed that ethanol soaking treatment could effectively delay color-changing, decrease decay incidence and water loss rate, and slow the decline of firmness to maintain the storage quality of papaya fruits. Moreover, ethanol treatment reduced the biosynthesis of MDA, ethylene, and superoxide anions, promoted the activities of SOD, CAT, and APX, and inhibited the activities of POD, PAL, and PPO, so as to hasten the antioxidant system and delay the maturation of postharvest papaya (Figure 6). In addition, that PCA showed that PAL, ethylene, and superoxide anions were the three most important indicators accounting for ripening and aging. In general, therefore, it seems that ethanol treatment has the potential of delaying the ripening and maintaining the storage quality of papaya fruits, and that treatment group B (25 mL/L ethanol) is the most suitable for papaya based on the TOPSIS analysis in this experiment. This study focused on physiological effects of ethanol treatment on papaya, and further studies should clarify the molecular regulation mechanism, in which RNA-seq could be performed for identifying main metabolism pathways and differentially expressed genes and choosing key genes for cloning and functional analysis.

DATA AVAILABILITY STATEMENT

The original contributions presented in this study are included in the article/supplementary material, further inquiries can be directed to the corresponding authors.

AUTHOR CONTRIBUTIONS

PC and ZL contributed to conception and designed the experiment. ZL, YM, and HL implemented the experiment. ZL processed and analyzed the data and wrote the first draft of the manuscript. FJ, PC, and HZ revised the manuscript and made modifications. PC supervised the whole study and provided guidance. PC and HZ acquired the funding. All authors have read and approved the manuscript.

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