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A study on the factors influencing the preservation rate of ascorbic acid in acerola cherry pulp

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Abstract

Acerola cherry grows in tropical regions. It contains a large amount of ascorbic acid (up to 4000 mg/100 g) but is not easy to preserve. It is very important to extend the preservation time of acerola cherries and maintain the ascorbic acid. Through single factor experiment and UV spectrophotometric method, the effect of five environmental factors (temperature, pH value, light, air, and metal ion) and four additives (sodium metabisulfite, EDTA, phytic acid and L-cysteine) on the content of ascorbic acid were investigated. Degradation curves of ascorbic acid and dehydroascorbic acid in acerola cherry pulp at 60°C were also explored. The results show that the influence of metal ions and air is far greater than other factors, the antioxidant effect of cysteine is higher than that of the other three additives and the degradation of dehydroascorbic acid occurred preferentially. This research provides theoretical support for the preservation of acerola cherry pulp.

Keywords: ascorbic acid; acerola cherry pulp; preservation; additives; degradation curve.

Practical Application: The acerola cherry fruit is perishable and unendurable to storage. Acerola cherry pulp is the raw material for a variety of acerola products. If the quality of acerola cherry pulp can be improved, it will be very beneficial to extend the preservation time and to process products. This research studied the degradation of ascorbic acid in acerola cherry pulp and the effects of various factors on the quality of acerola cherry pulp.

1 Introduction

With the development of society, people pay more and more attention to health. As the most well-known antioxidant, vitamin C has many functions such as whitening (Zerbinati et al., 2021), anti-cancer (Bakalova et al., 2020), senescence inhibition (Yang et al., 2018), and improving immunity (Luo et al., 2021). Vitamin C, also known as ascorbic acid, has strong reducing properties and is easily oxidized to dehydroascorbic acid. The reaction in this step is reversible. If dehydroascorbic acid is further hydrolyzed, diketogulonic acid is produced, which completely loses its physiological efficacy and the reaction is irreversible.

There are two types of common vitamin C supplements, one is artificially prepared vitamin C, and the other is natural fruits rich in vitamin C, such as kiwi, acerola and so on. Although the chemical structure is ascorbic acid, natural fruits also contain other biologically active substances, such as phenols and carotenoids. These active substances not only have their own antioxidant properties, but also have a synergistic effect with vitamin C to enhance the biological activity of vitamin C. Therefore, the vitamin C in natural fruits is easily absorbed and has high bioavailability. The benefits to people are greater (Chang et al., 2017).

The acerola cherry is the fruit of the acerola tree. Also known as Barbados cherries and West Indian cherries, the ripe fruits are bright red with thin skins. Acerola cherries are somewhat round and similar in size and shape to standard cherries but with three lobes. They have a sweet taste with a slight tartness. The fruit is valued for both its distinctive taste and its high vitamin C content. These small fruits contain a surprising amount of nutrients — along with potassium and magnesium, each acerola cherry can provide the daily vitamin C requirement.

However, freshly picked acerola cherries do not have much of a shelf life and are rarely grown for market. The acerola cherry tends to ferment, mold, or deteriorate within three to five days after picking. It is best eaten or processed as soon as possible after harvest. Processing methods, such as canning, preserve the cherries so they can be enjoyed at a later time. The fruit is often made into jams, jellies (Zhou et al., 2021), powder or juice (Luo et al., 2014). In addition to products made from acerola itself, extract of acerola acts as a natural antioxidant to improve the quality of other foods such as pork meat (Araújo et al., 2022). The preservation of acerola cherry pulp is easier than that of fresh fruit, and it can be used as a raw material for most acerola products. Extending the storage time of the acerola cherry pulp and maintaining the high quality of the acerola cherry pulp are vital to the production of acerola-related products. There is no research on how to improve the preservation effect of acerola cherry pulp.

This experiment studied the effects of five environmental factors (temperature, pH value, light, air, and metal ion) and four

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additives (sodium metabisulfite, EDTA, phytic acid and L-cysteine) on the content of ascorbic acid in acerola cherry pulp. At the same time, the content of ascorbic acid and dehydroascorbic acid and their degradation curves at 60 °C were studied. These studies provide a theoretical basis for improving the preservation effect of acerola cherry pulp.

2 Materials and methods

2.1 Materials

Acerola cherries were collected from the acerola cherry base (Dingan, Hainan Province, China) and preserved in the refrigerator freezer at -18 °C.Sodium metabisulfite and ethylenediaminetetraacetic acid (EDTA)were purchased from Sinopharm Chemical Reagent Co. Ltd.(Shanghai, China). Phytic acid and cysteine were procured from Shanghai Yuanye Biological Technology Co. Ltd. (Shanghai, China).Dehydroascorbic acid kit were obtained from Qiyi Biological Technology Co. Ltd. (Shanghai, China).

2.2 Methods

Effects of environmental conditions on the ascorbic acid content

Green and yellow acerola cherries were selected and mixed with 1% hydrochloric acid solution(v/v%) of equal weight. The mixture above was juiced into pulp as sample.

Acerola cherry pulp was stored at 5 °C, 25 °C, 35 °C, 45 °C and 65 °C for three hours, initial pulp was used as a control (CK).Ascorbic acid content of initial pulp and treated pulp was determined.

Disodium hydrogen phosphate-citrate buffer was added into pulp to adjust pH to 2.96, 3.20, 3,46, 3.67, 3.80, 4.10. Initial pH of pulp is 1.40 (Because the pulp contains 1% hydrochloric acid solution).The pulp was stored for one hour at room temperature (25 °C). The ascorbic acid content in treated pulp was determined.

1 mL 0.1 mol/L KCl, NaCl, $CaCl_2$, $MgSO_4$, $ZnSO_4$, $FeSO_4$, $CuSO_4$ and $AlCl_3$ solutions were added separately into eight groups. Distilled water was used as a control (CK). Ascorbic acid content in pulp was determined after four hours.

Pulp was divided into two groups. One group was given dark treatment and another control group was not. Ascorbic acid content in the pulp was determined at the same time everyday for seven consecutive days.

Pulp was divided into two groups. One group was given sealing treatment and the other group was not. Ascorbic acid content in the pulp was determined at the same time everyday for three consecutive days.

Effects of additives on the ascorbic acid content

Green and yellow acerola cherries were selected and mixed with 1% hydrochloric acid solution (v/v%) of equal weight. The mixture above was juiced into pulp as sample.

The effects of sodium metabisulfite, EDTA, phytic acid and cysteine on the AA in acerola cherry pulp were studied. Sodium metabisulfite with mass fraction of 0.04‰ to 0.08‰ were added to pulp series one. EDTA with mass fraction of 0.01‰ to 0.05‰ were added to pulp series two. Phytic acid with mass fraction of 0.12‰ to 0.28‰ were added to pulp series three. Cysteine with mass fraction of 0.2‰ to 1‰ were added to pulp series four. The choice of additive concentration is based on GB2760-2014-15 China (National Standard of the People's Republic of China, 2014). Then the treated samples were stored at 70 °C for one hour. The AA content in acerola cherries pulp was determined.

Total ascorbic acid content and analysis of composition

Acerola cherries were divided into green, yellow and red groups. Each group was squeezed into juice. Ascorbic acid content and dehydroascorbic acid content in the juice were determined.

Change of ascorbic acid concentrations and dehydroascorbic acid concentrations

Yellow acerola cherries were squeezed into juice. The juice was 60 °C water bath heat preserved in erlenmeyer flask. The changes of AA and DHAA concentrations were monitored up to 6 h.

Ascorbic acid content

The supernatant of pulp was token after the pulp was diluted.1ml supernatant and 9 mL 1% hydrochloric acid solution (v/v%) were well mixed. The determination of ascorbic acid content by spectrophotometry requires pH adjustment (Olgun et al., 2014). Ascorbic acid content was measured by spectrophotometrically at 245 nm (Shao et al., 2007; Wang et al., 2017) (Equation 1).

$$AA\ content\ (mg\ /\ 100g) = (c \cdot V) \cdot A \cdot 100 / 1000 \quad (1)$$

where c is the ascorbic acid concentration of the solution calculated according to ascorbic acid standard curve at 245 nm (μg/ml), V is the solution volume (10mL), ΔA is the diluted times of the pulp. 1000 is the conversion factor between μg/ml and mg/ml.100 is the conversion factor between g and 100g.

Dehydroascorbic acid content

The dehydroascorbic acid content is determined according to the instructions of the DHA kit (Equation 2).

$$\text{DHA content}(\text{mg} \cdot / \cdot \mathbf{100g} \cdot) = c \cdot \Delta \mathbf{A}_m / \Delta A_8 \cdot V_s \cdots A \cdot M \cdot 100 / 1000000 \quad (2)$$

where c is the dehydroascorbic acid concentration of the standard solution (100 μmol/L), ΔA_m is the difference in absorbance of test tube between 10s and 130s.ΔA_s is the difference in absorbance of standard tube between 10s and 130s.V is the solution volume of standard tube(0.1mL),A is the diluted times of the sample. M is the molar mass of dehydroascorbic acid (174.11 g/mol). 1000000 is the conversion factor between μg/L and mg/ml.100 is the conversion factor between g and 100g.

2.3 Analysis

SPSS22.0 (SPSS Inc., Chicago, IL, USA) was used to perform analysis of variance (ANOVA) to detect significant differences in data. $p < 0.05$ indicates a statistically significant difference.

The Duncan method is used when the homogeneity of variance is assumed, and the Tamhane's T2 method is used when the homogeneity of the variance is not assumed. Origin 2018 was used to draw graphs.

3 Result and discussion

3.1 Effects of environmental conditions on the ascorbic acid content

Figure 1 shows the effect of storage temperature on ascorbic acid content of acerola cherry pulp. The pulp can retain more ascorbic acid when stored at 5 °C. There is no significant difference ($p \geqq 0.05$) between the 5 °C group, 25 °C group and the control group. When storage temperature reaches 35 °C, the content of ascorbic acid is lower. When storage temperature reaches 65 °C, the content of ascorbic acid decreased drastically. The heat treatment may degrade ascorbic acid by non-enzymatic reactions. The decrease in content of ascorbic acid are more extensive at higher processing temperatures (Martinsen et al., 2020). On the other hand, when two heat-sensitive compounds such as anthocyanins and ascorbic acid are present simultaneously, the presence of ascorbic acid will protect the anthocyanins to a great extent. Thus, the anthocyanin can still maintain a high level after thermal processing(Grigio et al., 2022). Under low temperature storage conditions, the main reason for the higher ascorbic acid content is low temperature storage conditions enhanced the expression of most genes coding for enzymes involved in L-Ascorbic acid biosynthesis and redox reactions (Tsaniklidis et al., 2014). If acerola cherries are stored at a lower temperature than 5 °C, on the one hand, it cannot improve the preservation rate of ascorbic acid, and on the other hand, it will increase the storage cost.

Figure 2 shows the effect of pH value on ascorbic acid content of acerola cherry pulp. Generally, the pH value of acerola cherry pulp ranges from 3.2 to 3.5.Group of pH 3.46 has higher content of ascorbic acid than other groups ($p < 0.05$). The pH value is exactly in the range of 3.2 to 3.5, so it is inferred that maintaining the original pH value of the pulp is more conducive to the preservation of ascorbic acid. A lower pH also maintains a deeper red color, which may be related to the anthocyanins in acerola cherries, and gellan gum can further increase the stability of the pigment (Leal et al., 2022). Acidic conditions are conducive to the preservation of ascorbic acid. However, for acerola cherry pulp, other fruit pulps and ascorbic acid solutions, the pH value that is most conducive to the preservation of ascorbic acid is different. Because in addition to ascorbic acid, fruit pulp contains polyphenol antioxidants such as flavonoids and anthocyanins. These components not only affect the preservation of ascorbic acid, but also affect the pH value.

Figure 3 indicates the effects of metal ions on content of ascorbic acid in acerola cherry pulp. Fe^{2+} and Cu^{2+}catalyze oxidation reaction of ascorbic acid (Mayadevi et al., 1998; Sailani et al., 2011), so the content of ascorbic acid in these two

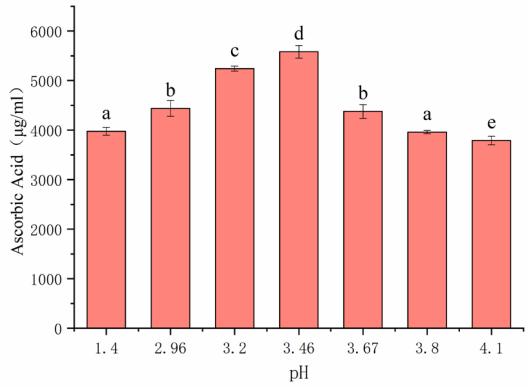

Figure 2. The effect of different pH values (one hour storage at 25℃) on ascorbic acid content of acerola cherry pulp. Analysis of variance followed by the Duncan's test and Tamhame's test. Values with different letters at the different pH values are significantly different ($p < 0.05$).

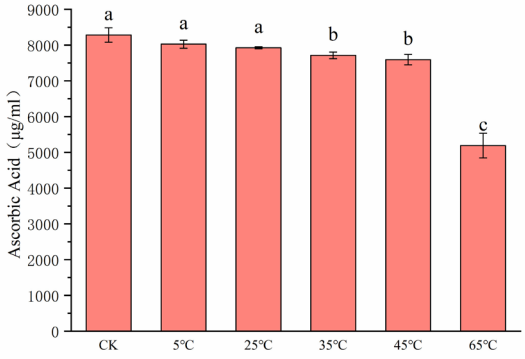

Figure 1. The effect of different storage temperatures (three hours storage) on ascorbic acid content of acerola cherry pulp. CK: Initial ascorbic acid content was tested in this group. Analysis of variance followed by the Duncan's test and Tamhame's test. Values with different letters at the different storage temperatures are significantly different ($p < 0.05$).

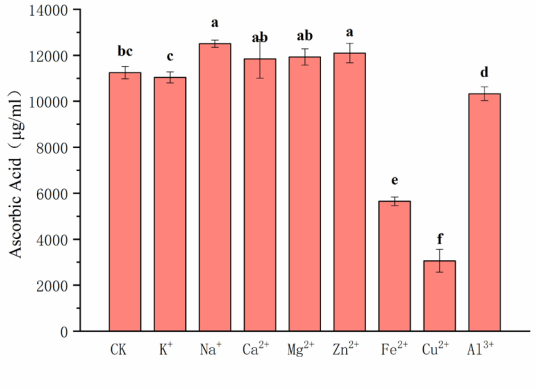

Figure 3. The effect of different metal ions (four hours storage at 25℃) on ascorbic acid content of acerola cherry pulp. CK: Replace the metal ion solution with the same amount of distilled water. Analysis of variance followed by the Duncan's test and Tamhame's test. Values with different letters at the different storage temperatures are significantly different ($p < 0.05$).

group is lowest. Catalysis of Fe^{3+} is even stronger than Cu^{2+} (Barbosa et al., 2012). K^{+} and Al^{3+} make content of ascorbic acid lower. Na^{+}, Ca^{2+}, $Mg^{2+}$and Zn^{2+} have no impact or even increase the content of ascorbic acid. Because metal elements such as sodium, magnesium and calcium are more active than copper and iron, their cationic oxidizing properties is not as strong as copper ion and iron ion.

Figure 4 displays the effect of light on ascorbic acid content of acerola cherry pulp. The loss rate of ascorbic acid in cherries without dark treatment (31.64%) is significantly higher than that of cherries with dark treatment (11.26%). The effect of light on the oxidation reaction of ascorbic acid is continuous, and the ascorbic acid content steadily decreases in seven days. Oxidation of pulp during light storage causes a significant loss of vitamins and produces off-flavors (Hall et al., 2010). Optical oxidation is carried out by irradiating oxidants to generate free radicals with strong oxidizing ability (·OH) under the radiation of light. The generated free radicals undergo a dehydrogenation reaction with ascorbic acid.

Figure 5 illustrates the effect of air on ascorbic acid content of acerola cherry pulp. The loss rate of ascorbic acid in cherries without sealing treatment (40.48%) is significantly higher than that of cherries with sealing treatment (6.53%).In addition, microorganisms, especially molds, will multiply quickly in cherries without sealing treatment. The influence of air or oxygen on the oxidation reaction of ascorbic acid is higher than that of light. On the one hand, the oxygen in the air provides oxygen free radicals for the oxidation reaction. On the other hand, exposure of the acerola cherry pulp to the air causes the mold to multiply and produce toxins to contaminate the pulp.

3.2 Effects of additives on the ascorbic acid content

Effects of additives on the ascorbic acid content of acerola cherry pulp are shown in Figure 6. As Figure 6a showed, adding sodium metabisulfite to acerola cherry pulp will increase the preservation rate of ascorbic acid. When the concentration of sodium metabisulfite increases, the preservation rate of ascorbic acid does not change significantly. When the mass fraction of sodium metabisulfite is 0.05‰, the preservation rate of ascorbic acid in the pulp is higher and the amount of additive is less. Sodium metabisulfite is a strong reducing agent, it is oxidized when exposed to oxygen, thereby protecting other easily oxidizable components. In addition, sodium metabisulfite can inhibit the growth of microorganisms and prevent the rapid rise in the microbial population of pulp resulted in biomass deterioration and substantial dry matter loss and sugar exhaustion (Ahmadi et al., 2021).

As Figure 6b showed, adding EDTA to acerola cherry pulp will increase the preservation rate of ascorbic acid. When the concentration of EDTA increases, the preservation rate of ascorbic acid does not change significantly. When the mass fraction of EDTA is 0.02‰, the preservation rate of ascorbic acid in the pulp is higher and the amount of additive is less. The preservation effect of EDTA is not as obvious as sodium metabisulfite. Metal ions can catalyze the auto-oxidation reaction. EDTA chelates with metal ions to prevent metal ions from catalyzing the auto-

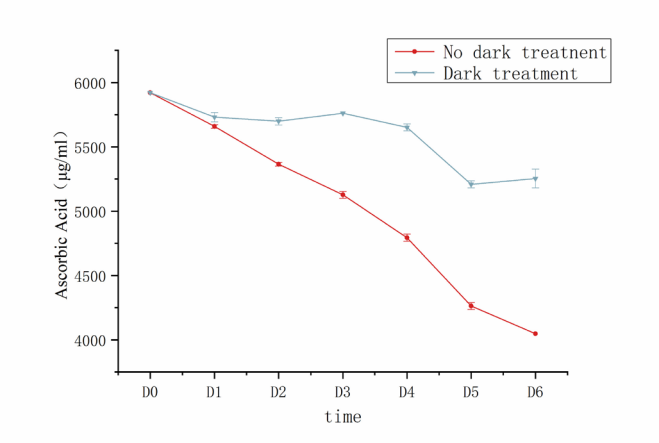

Figure 4. The effect of light (7 days storage at 25°C) on ascorbic acid content of acerola cherry pulp.

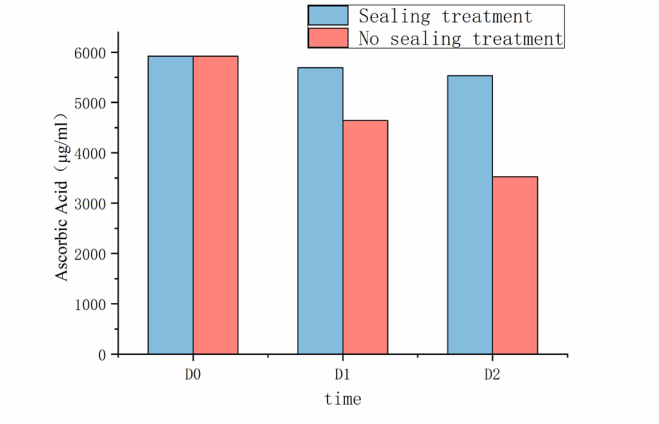

Figure 5. The effect of air (three days storage at 25°C) on ascorbic acid content of acerola cherry pulp.

oxidation reaction of substances. In this experiment, the effect of EDTA is not significant. There may be two reasons. First, there are no metal ions in the environment itself. Second, the effect of EDTA under acidic conditions is not as significant as under neutral conditions. Acerola cherry pulp is an acidic solution (pH=3.4), which limits the effect of EDTA (Xu et al., 2013).

As Figure 6c showed, adding phytic acid to acerola cherry pulp will reduce the preservation rate of ascorbic acid. When the concentration of phytic acid increases, the preservation rate of ascorbic acid continues to decrease. When phytic acid is not added, the preservation rate of ascorbic acid in the pulp is higher. The preservation effect of phytic acid is even negative. This result is hard to imagine, and it is completely opposite to the results of other similar experiments (Song et al., 2020). There are two types of anti-oxidant mechanisms of phytic acid: one type is capable of ionizing hydrogen ions and reacting with free radicals, thereby destroying the peroxide produced in the process of self-oxidation, making it unable to continue to form products

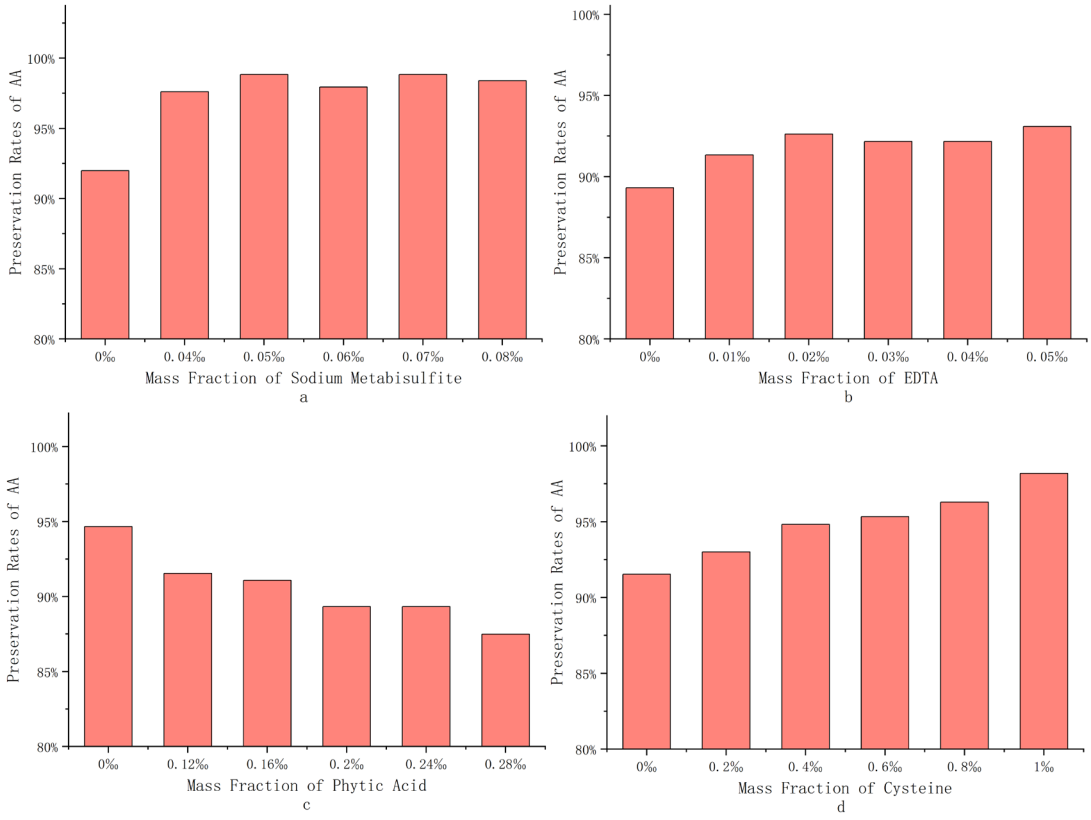

Figure 6. Effects of four additives (one hour storage at 70℃) on preservation rate of ascorbic acid in acerola cherry pulp.

such as aldehydes and ketones. The other is that it has a strong chelating ability, which can make the multivalent metal ion that promote oxidation is chelated into a stable chelate, so that it loses the effect of promoting oxidation. Although phytic acid is a common antioxidant, a single addition of phytic acid may have the effect of promoting oxidation, and there are literatures mentioning that phytic acid can accelerate browning. Ascorbic acid can also promote oxidation under certain conditions, but the principle is not yet clear (Hao, 2002; Nachtigall et al., 2010).

As Figure 6d showed, adding cysteine to acerola cherry pulp will increase the preservation rate of ascorbic acid. When the concentration of cysteine increases, the preservation rate of ascorbic acid continues to increase. Even if the mass fraction of cysteine is increased to 1‰, the preservation rate of ascorbic acid in the pulp is still increasing. The reason why the preservation effect of cysteine is so excellent is that the sulfhydryl group in L-cysteine has strong reducing properties, and the redox potential of L-cysteine is lower than that of ascorbic acid. It is conceivable that if you continue to increase the amount of cysteine, the preservation rate of ascorbic acid can exceed 100%. However, the amount of cysteine is restricted by the standard GB2760-2014-15 (National Standard of the People's Republic of China, 2014) and the production cost should be considered.

3.3 Total ascorbic acid content and analysis of composition

Total ascorbic acid composition of cherries with different maturities is shown in Table 1. There is no significant difference in the content of dehydroascorbic acid in fresh acerola cherries of different maturity. The main reason for the difference in the content of total ascorbic acid is that the content of ascorbic acid in fresh acerola cherries of different maturity is quite different. In mature acerola cherries, the content of ascorbic acid accounts for 44.73% of the total ascorbic acid content. In half-mature acerola cherries, this proportion is 47.11%. In immature acerola cherries, this proportion is 49.59%. During the ripening process, the acid in the fruit is used as a raw material to synthesize carbohydrates. In addition to ascorbic acid, polyphenols, flavonoids and pigment content also change during the maturation process (Shah et al., 2020). Most of differential accumulated amino acids, flavonoids, lipids, and terpenoids predominantly accumulated in the mature fruits and ascorbic acid predominantly accumulated in the immature fruits (Xu et al., 2021). Acerola cherries with low maturity have stronger antioxidant capacity. Different varieties of acerola have different types of organic acids and antioxidant capacity(Guedes et al., 2022). Therefore, the selection of which maturity level and variety of acerola cherries for deep processing needs to be considered from various aspects.

3.4 Change of ascorbic acid concentrations and dehydroascorbic acid concentrations

High temperature can accelerate the oxidation reaction, which convert ascorbic acid to dehydroascorbic acid and convert dehydroascorbic acid to diketogulonic acid .Ascorbic acid and dehydroascorbic acid degradation kinetic curve of acerola cherry

Table 1. Ascorbic acid content and dehydroascorbic acid content in acerola cherries with different maturities.

Maturity	AA (mg/100 g)	DHAA (mg/100 g)	AA/(AA+DHAA) (%)
Mature	1996 ± 19.75	2467 ± 29.62	44.73
Half-mature	2133 ± 26.74	2394 ± 102.6	47.11
Immature	2522 ± 7.290	2563 ± 446.4	49.59

expressed as the mean of three replicates ± standard deviation.

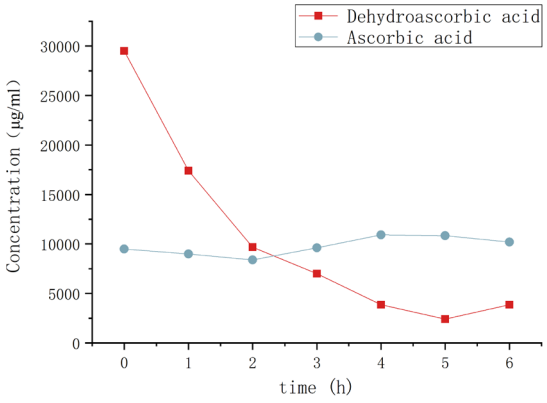

Figure 7. Ascorbic acid and dehydroascorbic acid degradation kinetic curve of acerola cherry juice (6 hours at 60℃).

juice at 60 °C are shown in Figure 7. During the six-hour heating process, ascorbic acid changes relatively smoothly, first falling, then rising, and then falling. The final ascorbic acid content is slightly higher than the initial value. The change of dehydroascorbic acid was dramatic. Its content continued to decrease in the first five hours and only increased slightly in the sixth hour. The change in total ascorbic acid content is a combination of the above two processes. Its content continued to decrease in the first four hours and was stable in the last two hours. At the above-mentioned temperature and initial concentration, the two-step oxidation reaction preferentially oxidizes dehydroascorbic acid, and gradually reaches equilibrium after 6 hours. This is different from previous studies on the degradation kinetics of ascorbic acid (Serpen & Gökmen, 2007). In their experiment, the content of dehydroascorbic acid increased first and then decreased, and the content of ascorbic acid continued to decrease. First, the initial concentration ratio of ascorbic acid and dehydroascorbic acid is different. Second, natural fruit pulp and artificially prepared solutions are also different.

4 Conclusion

In this study, the effects of environmental factors such as temperature, pH value, light, air, and metal ions on the content of ascorbic acid in acerola cherry pulp were investigated. Acerola cherry pulp is suitable for storing at the condition that the temperature is 5 °C, pH value is range from 3.2 to 3.5, no light, sealed, and without Fe^{2+}, Fe^{3+}, Cu^{+}, Cu^{2+} and Al^{3+}. In addition, the effect of additives on the content of ascorbic acid in acerola cherry pulp has also been explored. Sodium metabisulfite and L-cysteine can greatly reduce the loss rate of ascorbic acid in the pulp. The content of ascorbic acid and dehydroascorbic acid in acerola cherry pulp was also determined. The content of ascorbic acid was slightly less than that of dehydroascorbic acid, and the content of ascorbic acid reduced with the raise of maturity. The degradation kinetics of ascorbic acid and dehydroascorbic acid under heating at 60°C was also examined, and it was found that the degradation of dehydroascorbic acid occurred preferentially in the first six hours. This research provides theoretical support for the preservation of acerola cherry pulp and the pretreatment of acerola cherry products.

Conflict of interest

The authors declared no conflicts of interest.

Acknowledgements

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