Effects of different methods on reducing acid of papaya juice and papaya wine

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Abstract. Effects of different acid-reducing methods on the deacidification effect of papaya fruit juice and wine, thought as raw materials, were studied. The papaya juice was treated with two methods, calcium carbonate, calcium carbonate combining with biological acid-reducing. Calcium carbonates of the first method were 7.0 g/L, 8.0 g/L, 9.0 g/L and 10.0 g/L, respectively. Whereas the second method was that pulped Papaya raw material was added 22.38 g/L calcium carbonate. After 24 hours of quiescence, 1 g/100 kg of lactic acid bacteria was also added. The acid-reducing effect was expressed by titration acid. The wine was tested with calcium carbonate (8.05 g/L, 9.05 g/L and 10.05 g/L, in turn) and potassium tartrate (19.45 g/L, 20.45 g/L and 21.45 g/L, in turn). The acidity reduction effects was also showed by titration acid. Two methods could all reduce acid content of Papaya juice, but effect of calcium carbonate on acid-reducing was more obvious with an average decrease of 11.25 g/L. Two methods could reduce all the acid content of the wine, but the effect of calcium carbonate was also the most significant with an average reduction of 12.0 g/L. The second was potassium tartrate. In general, calcium carbonate was time-consuming and easy to achieve acid-lowering purpose, and the second was less and low cost to calcium carbonate. Effect of 9.0 g/L and 10.05 g/L calcium carbonate to reduce acid in papaya juice and papaya wine was the most obvious with short time and low cost. Therefore, 9.0 g/L and 10.05 g/L of calcium carbonate were the best choice to reduce the acid content of papaya juice and papaya wine with higher acid content, respectively.

1. Introduction
Papaya has health care functions. The products that have been successfully developed were mainly papaya juice beverage, papaya preserves, papaya wine, papaya vinegar and so on. Papaya contained also better nutritional components, for example protein-decomposing enzymes, carotene and rich vitamin C[2]. Li et al. [3] analyzed the organic acids in papaya with GC-MS and HPLC. The results showed that there were 22 kinds of abundantorganic acids in papaya, accounting for 94.63% of the total content, which were mainly fatty acid, dibasic acid, ternary acid and a small amount of aromatic acid. The sum of relative contents of palmitic acid and linoleic acid was 39.87%. The relative content
The highest content of malic acid was 14.20%. The highest content of malic acid was found in papaya, 29.72 g/L, accounting for 81.2% of the organic acid content.

To make full use of the active ingredients of papaya and improve the economic benefits of the industry, papaya wine can be directly fermented by papaya. The content of sugar in papaya was low, whereas the acid content was higher, and the taste of papaya fruit wine was sharp and rough. Therefore, in order to reduce the original acid content of papaya and improve its quality, an effective deacidification method to keep the original flavor of papaya wine was more important.

At present, there were three methods to reduce acid in fruit wine at home and abroad, which was chemical method, physical method and biological method [4-7]. Calcium carbonate and potassium tartrate are used as basic chemical substances to reduce the acid content. However, this method would affect the taste and color of wine, resulting in loss of light and turbidity, and even reduce the quality of the wine [8]. Physical method includes cold treatment and ion exchange method. This method can reduce the acid content of fruit juice and wine without introducing other ingredients and influence on the quality of wine, but there was not obvious effect and longer time. Biological acid-lowering method, malic acid-lactic acid fermentation, was also time-consuming and difficult to control in practical production [9-10].

The raw material of papaya in this experiment comes from Lincang City, Yunnan Province. Papaya fruit has a high acid content including wine fermented from papaya. In order to ensure the taste of wine, method of this experiment was that reducing firstly the acid content of papaya juice, so that yeast can grow and reproduce under suitable conditions, in order to achieve the best fermentation effect. In order to solve the problem of sour and astringent taste producing high quality papaya wine, the papaya wine was treated again.

2. Materials and methods

2.1. Materials
Papaya juice and papaya fruit wine were brewed in the Grape and Wine Technology Laboratory of Chemistry and Life Sciences College of Chuxiong Normal University in April 2018.

Papaya juice was added 22.38 g/L calcium carbonate to decrease acid for 24 h, before adding sugar and corn sugar respectively to start-up fermentation.

2.2. Design 1: Fruit juice decreases acidity
Deacidification of Calcium Carbonate: 100 mL papaya juice, including 7.0 g/L, 8.0 g/L, 9.0 g/L and 10.0 g/L calcium carbonate, was added into 250 mL triangular bottle, respectively, meanwhile stirred for 1 to 2 hours to dissolved fully. Titrated acid was determined after 2 and 24 hours.

Carbonate + Biological Acid-reducing: After acidity of papaya juice was measured by titration, 22.38 g/L of calcium carbonate was added to reach 16.88 g/L titrative acid. After 24 hours of treatment, which was 12.38 g/L. Finally, 1 g/100 kg lactic acid was added. After that, the closed oxygen-insulated fermentation was carried out, and titratable acid was monitored every day until it remains unchanged for three days.

2.3. Design 2: Fruit wine decreases acidity
Acid-reducing of Calcium Carbonate: Taking 100 mL wine, including 8.05 g/L, 9.05 g/L and 10.05 g/L calcium carbonate, into 250 mL triangular bottle, respectively, meanwhile stirred for 1 to 2 hours to dissolved fully. Titrated acid was determined after 2 and 24 hours.

Acid-reducing of Potassium Tartrate: Taking 100 mL wine, including 19.45 g/L, 20.45 g/L and 21.45 g/L potassium tartrate, into 250 mL triangular bottle, respectively, meanwhile stirred for 1 to 2 hours to dissolved fully. Titrated acid was determined after 2 and 24 hours.
2.4. Experimental method
According to GB/T 15038-2006 [11], the content of titrable acid was determined by acid-base titration.

2.5. Statistical Analysis
Data analysis was conducted using Excel 2013.

3. Results and discussions
3.1. Effect of different amount of calcium carbonate on acid reducing effect of papaya juice
The acid reducing effect of calcium carbonate on papaya juice was shown in Table 1.

| TABLE 1. The effect of different calcium carbonate on the acid reduction of papaya juice |
|-----------------------------------------------|------------------------|------------------------|------------------------|------------------------|------------------------|
| Calcium carbonate (g/L)                       | 7.0 (g/L)              | 8.0 (g/L)              | 9.0 (g/L)              | 10.0 (g/L)             |
| 2 h                                           | 39.18±1.23             | 28.68±1.33             | 28.31±1.36             | 27.56±1.32             | 27.18±1.45             |
| 24 h                                          | 23.06±1.74             | 21.56±1.62             | 20.81±1.22             | 20.81±1.76             |

The acid-reducing effect was indicated by titrating acid (g/L). Table 1 showed that with the increasing amount of calcium carbonate, the titrating acid gradually decreased, which was consistent with the research of Liu et al. [12]. Compared to the beginning value, addition of calcium carbonate could decrease titration acid content by 10.50 g/L, 10.87 g/L, 11.62 g/L and 12.00 g/L after 2 hours, respectively. While after 24 hours, its content decreased by 16.12 g/L, 17.62 g/L, 18.37 g/L and 18.37 g/L, respectively. At the same dosage at different times, the titration acid content decreased 5.62 g/L, 6.75 g/L, 6.75 g/L and 6.75 g/L, respectively. At the same dosage at different times, the titration acid reach 20.81 g/L after 24 hours. Therefore, In order to save cost, 9.0 g/L calcium carbonate was selected to reduce acid in papaya juice. Through analysis, with increasing the amount of calcium carbonate, there should be enough time to achieve the effect of reducing acid. Sun et al.[13] studied that calcium carbonate could reduce acid conveniently, time-consuming and low-cost. Calcium carbonate + biological method to reduce acid.

Table 2 showed reducing acid effect of calcium carbonate and biological method on papaya juice.

| Table 2. Analysis of Reducing Acid Effect of Calcium Carbonate Combined With Biological Method |
|-----------------------------------------------|------------------------|------------------------|------------------------|------------------------|
| Time                                          | Adding sugar cane pH   | Titrating acid (g/L)   | Adding saccharification solution pH | Titrating acid (g/L)   |
| 0 h                                           | 2.87±0.23              | 46.88±1.32             | 2.87±0.45              | 46.88±2.22             |
| 24 h                                          | 4.38±0.33              | 12.38±2.01             | 4.38±0.02              | 12.38±1.32             |
| First days                                    | 4.60±0.23              | 10.31±1.56             | 4.60±0.12              | 10.31±1.56             |
| Second days                                   | 4.46±0.25              | 8.63±1.32              | 4.45±0.10              | 8.63±1.85              |
| Third days                                    | 4.44±0.32              | 8.63±1.42              | 4.42±0.11              | 8.63±1.46              |
| Fourth days                                   | 4.45±0.22              | 8.63±1.65              | 4.46±0.24              | 8.63±1.32              |
| Fifth days                                    | 4.38±0.66              | 8.63±1.36              | 4.38±0.45              | 8.63±1.25              |
| Sixth days                                    | 4.44±0.45              | 8.63±1.22              | 4.37±0.55              | 8.63±1.39              |

Note: Papaya raw materials were pulped, soaked in cold for 24 hours, and then the skin and slag were separated and filtered. 22.38 g/L calcium carbonate was added, and the precipitate was filtered after 24 hours. Then, 1 g/100 kg lactobacillus was added. Titration acid and pH were determined on the first day of fermentation.

The acid-reducing effect was indicated by titrating acid (g/L). Table 2 showed that the material titrating acid was 46.88 g/L, while the value decreased by 36.57 g/L after 24 hours adding 22.38 g/L calcium carbonate and stirring for 1 to 2 hours. Then, 1 g/100 kg lactic acid bacteria was added for
fermentation. For the first day, titrating acid of papaya juice was 10.31 g/L with the original fragrance of papaya. Then it was kept 8.63 g/L for 4 days of continuous fermentation. After that, there was a slight milky odor. Wang and Zhang [14] researched that the optimum pH for lactic acid growth was 5.5. As the pH goes up, the growth of lactic acid bacteria was accelerated between 3.0 and 5.5. The experiment found that when the pH was about 4.3, the titrative acid content remained unchanged at 8.63 g/L.

3.2. Effect of different amount of calcium carbonate on acid-reducing effect of papaya wine
Table 3 showed effect of calcium carbonate on reducing acid of papaya wine.

| Time | 0 (g/L) | 8.05 (g/L) | 9.05 (g/L) | 10.05 (g/L) |
|------|---------|------------|------------|------------|
| pH   | Titrating acid (g/L) | pH   | Titrating acid (g/L) | pH   | Titrating acid (g/L) | pH   | Titrating acid (g/L) |
| 2 h  | 3.40 ± 0.26 | 4.36 ± 0.67 | 7.86 ± 1.38 | 4.43 ± 0.32 | 7.12 ± 1.32 | 4.49 ± 0.37 | 6.38 ± 1.05 |
| 4 h  | 0.26 ± 0.56 | 4.40 ± 0.76 | 7.12 ± 1.32 | 4.50 ± 0.42 | 7.12 ± 1.48 | 4.56 ± 0.56 | 5.63 ± 1.38 |
| 24 h | 4.47 ± 0.36 | 7.12 ± 1.32 | 4.62 ± 1.32 | 6.38 ± 1.32 | 4.72 ± 0.56 | 5.63 ± 1.38 |

The acid reducing effect was indicated by titrating acid (g/L) and pH. Table 3 showed with the increase of calcium carbonate dosage, titrative acid decreased gradually after 2 hours. A subtle change had taken place after 4 hours. 8.05 g/L and 9.05 g/L of calcium carbonate reduced the titrative acid by 7.86 g/L. After 24 hours, the titrative acid was decreasing also gradually with the increase of dosage. The titration acid was reduced to 7.12 g/L by using 8.05 g/L calcium carbonate for 2 h, which was reduced to 7.12 g/L again after 4 h and 24 h. However, the titration acid was reduced to 7.12 g/L by using 9.05 g/L calcium carbonate for 2 h and 4 h, which was 6.83 g/L after 24 h. The titration acid was reduced to 6.38 g/L by using 10.05 g/L calcium carbonate for 2 h, which was all 5.63 g/L after 4 h and 24 h. The pH value increased with the increase of calcium carbonate dosage, and the acid-reducing time also increased. Sun et al. [13] found that calcium carbonate was convenient to reduce acid with short time-consuming and low-cost. Therefore, the final choice of 10.05 g/L calcium carbonate for acidity reduction of papaya wine owe to the obvious effect of acidity reduction after 2 hours, saving time and cost.

3.3. Acid-reducing effect of different amount of potassium tartrate on papaya wine
Table 4 showed effect of potassium tartrate on reducing acid of papaya wine.

| Time | 0 (g/L) | 19.45 (g/L) | 20.45 (g/L) | 21.45 (g/L) |
|------|---------|------------|------------|------------|
| pH   | Titrating acid (g/L) | pH   | Titrating acid (g/L) | pH   | Titrating acid (g/L) | pH   | Titrating acid (g/L) |
| 2 h  | 3.99 ± 0.26 | 16.59 ± 1.32 | 4.00 ± 1.32 | 16.88 ± 1.32 | 4.03 ± 1.32 | 15.38 ± 1.32 |
| 4 h  | 0.56 ± 0.67 | 1.59 ± 1.59 | 0.22 ± 1.59 | 1.59 ± 1.59 | 0.28 ± 1.59 | 1.46 ± 1.59 |
| 24 h | 3.99 ± 0.36 | 16.12 ± 1.32 | 4.00 ± 1.32 | 16.12 ± 1.32 | 4.04 ± 1.32 | 15.38 ± 1.32 |

The acid-reducing effect was indicated by titrating acid (g/L) and pH. Table 4 showed that with the increase of potassium tartrate dosage after 2 hours and 4 hours, titrative acid decreased gradually. 19.45 g/L, 20.45 g/L and 21.45 g/L of potassium tartrate could not reduce acid after 2 h and 4 h, which
was 17.63 g/L, 16.88 g/L and 15.38 g/L, respectively. While after 24 hours, 19.45 g/L and 20.45 g/L of potassium tartrate made the titration acid keep 16.12 g/L. From contrast, titration acid of papaya wine was determined all by 15.38 g/L under potassium tartrate with 21.45 g/L after 2 h, 4 h and 24 h to, respectively. Through analysis, the effect of time limit on reducing acid was not obvious when potassium tartrate dosage was increased. It was also found that the titrating acid was 15.38 g/L when potassium tartrate of 21.45 g/L was used. The decrease of titrating acid was also small at 2 h and 4 h. Therefore, according to this test, potassium tartrate was not suitable for reducing acid in papaya wine. Liu et al. [12] found that dosage of potassium tartrate to reduce acid was too larger and the cost was higher, which could combine with other acid-reducing agents for acid-reducing test. The pH value increased also with the increase of calcium carbonate dosage and acid-reducing time.

Calcium carbonate, potassium bicarbonate, potassium tartrate and double salt were commonly used to chemical drop acid in the production. Chemical acid reducer had the advantages of easy operation, easy control and obvious effect of acid reduction [14]. Calcium carbonate had the advantages of quick reaction, low cost and convenient operation. Potassium tartrate could reduce acid by adjusting the composition of tartrate in wine, and there was no gas in the reaction process, which was an internal reaction process [14]. The results displayed that the effect of calcium carbonate on reducing acid of papaya fruit juice and wine was obvious and it took less time. Papaya juice also had the fragrance of papaya after reducing acid with calcium carbonate, while papaya wine was slightly absent in aroma, but in general it also had the fragrance of papaya without other odors. Potassium tartrate had a certain effect on reducing acid content in fruit wine, but white precipitation was found during the treatment, and milky white was also observed when the wine was shaken slightly. This phenomenon needed to be studied further. Comparing with calcium carbonate in reducing acid, it was found that papaya wine had lost the fragrance of papaya at this time. Researches of Ding [15] showed that potassium tartrate had the characteristic of larger reducing-acid dosage, and easy to produce potassium hydrogen tartrate, not easy to remove. These phenomena were caused by the presence of other salts in liquor, such as sulfate, potassium salt and tartrate. Potassium tartrate was added into wine to reduce acid content, and some potassium ions were introduced artificially.

At this time, acid radical ions and potassium ions could form complex deposits and precipitate. This phenomenon indicated the precipitation formed by the combination of acid ion and potassium ion. Xue [16] found that when the pH value of the solution was 3.7, the hydrogen ion of tartrate accounted for the largest proportion, which was easy to produce precipitation of potassium hydrogen tartrate. When calcium carbonate was combined with biological acid to low to reduce acid, it was found that the titration acid content did not change for 3 consecutive days and the titration acid content was 8.63 g/L, but the acid content was reduced by 1.68 g/L compared with that before fermentation, and no change occurred after that. However, this method showed bad phenomena in aroma. After no change in titrative acid, the aroma showed milky odor and the original color of papaya was absent. Peynaud and Maurié [17] showed that malolactic fermentation could reduce total acid by 1 - 3 g/L. After the addition of lactic acid bacteria, it was found that the titration acid decreased from 10.31 g/L to 8.63 g/L, with a decrease of 1.68 g/L, which was consistent with the study of Peynaud and Maurié [17].

To make a wine with papaya as raw material, reducing acid was an essential operation step, because the high acid content would affect the later fermentation, yeast could not reproduce in the normal environment. Through the comparison with acid-reducing effect of papaya wine, it was found that the use of calcium carbonate could reduce effectively the acid in wine, and achieve the distinct effect in a short time. However, the effect of potassium tartrate on reducing acid was not obvious, with white precipitate. The wine was generally milky white when shaken slightly, but other factors could not be excluded, for example the presence of acid reacting rarely with potassium tartrate in papaya wine, or the fact that potassium tartrate alone was not suitable for reducing acid in papaya wine, which needed further research.
4. Conclusions
In this study, calcium carbonate was finally used to reduce acid in papaya juice and papaya wine respectively, with the dosage of 9.0 g/L and 10.05 g/L respectively. Compared with potassium tartrate, calcium carbonate had the advantages of fast reaction, low cost and convenient use. Calcium carbonate could reduce acid in papaya juice to achieve quickly the effect of reducing acid, and retain the original fragrance of papaya. At the same time, calcium carbonate could reduce acid in papaya juice, however affect the color of the wine, aroma loss was small. Unlike potassium tartrate with appearance of a white precipitate and milky white after slightly shaking when reducing acid. It was not recommended to use calcium carbonate and biological to reduce acid, because the combination of the two was not obvious with a certain risk.

Acknowledgments
This research fund was funded by the 2017 National College Students’ Innovation and Entrepreneurship Training Program (17007), Engineering Technology Research Center of Grape and Wine for advanced school in Yunnan, 12th Five - Year Degree Authorized Construction Discipline in Biology of Yunnan Province, University - level Academic Backbone Training Project for Chuxiong Normal College (XJGG1603), Youth Project of Yunnan Applied Basic Research Projects (2016FD088), Surface Project of Joint Special Fund Project on Basic Research for Local Undergraduate Colleges and Universities (part) in Yunnan province (2018FH001-043).

References
[1] Bai Z C, Liu S Y and Zhou Z Q 2009 China Agricultural Press 1 69.
[2] Wang J X 2005 Food Sci. 26 208.
[3] Li Q, Liu L Q, Xu H D, Liu L P and You X X 2008 J. Northwest Agric. 17 207.
[4] Vera E, Sandeaux J, Persin F, Pourcelly G, Dormier M and Ruales J 2007 J. Food Eng. 78 1427.
[5] Calle E V, Ruales J, Dormier M, Sandeaux J, Sandeaux R and Pourcelly G 2002 Desalination. 149 357.
[6] Sumby K M, Jiranek V and Grbin P R 2012 Food Chem. 141 1673.
[7] Vera E, Ruales J, Dormier M, Sandeaux J, Persin F, Pourcelly G and Reynes M 2003 J. Food Eng. 59 361.
[8] Wen L K, Zhao W, Zhang W and Fu Y H 2010 Food Sci. 11 325.
[9] Genisheva Z, Mussatto SI, Oliveira JM and Teixeira JA 2012 Food Chem. 128 1510.
[10] Gamella M, Campuzano S, Conzu elo F, Curiel JA, Muñoz R, Reviejo AJ and Pingarrón JM, Talanta 81 925.
[11] National standard of the People's Republic of China. 2006 GB/T15038-2006 general analysis method for wine and fruit wine. China Standard Press.
[12] Liu S, Liu Q R, Yuan F and Gao Y X 2014 Food Sci. Tech. 39 83.
[13] Sun H H, Yan X H, Zhang J P, Xu G J and Chen M B 2012 J. I. Brewing. 40 27.
[14] Wang H and Zhang C H 1996 J. Northwest Agric. University. 24 92.
[15] Ding J 2009 Anhui Agric. Sci. Bulletin. 15 198.
[16] Xue J 2002, Sino-overseas Grapevine Wine 5 54.
[17] Peynaud E and Mauriè A 1958 Am. J. Enol. Viticul. 9, 32.