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Yingxue Zhong; Xiaofang Zhang; Ruiqun Yang; Fang Tao; Yashan Li; Chengdong Xu; Lijun Nan ✉; Jing Huang ✉

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Clarifying Effect of Different Clarifying Agents on Chaenomeles Juice and Wine

Yingxue Zhong[1, a)], Xiaofang Zhang[1, a)], Ruiqun Yang[1], Fang Tao[1], Yashan Li[1,2], Chengdong Xu[1,2], Lijun Nan[1, 2, b)] and Jing Huang[3, c)]

[1]*School of Chemistry and Life Sciences, Chuxiong Normal University, Chuxiong 675000, China*
[2]*Engineering Technology Research Center of Grape and Wine for advanced school in Yunnan, Chuxiong Normal University, Chuxiong 675000, China*
[3]*School of Mathematics and Statistics, Chuxiong Normal University, Chuxiong 675000, China*

[a)]Yingxue Zhong and Xiaofang Zhang contributed equally to this work and should be considered co-first authors.
[b)]Corresponding author: submit_paper73@126.com
[c)]Corresponding author: jing_huang12@126.com

Abstract. The turbidity and precipitation affect seriously sensory quality of chaenomeles juice and wine. In order to reduce the harmful effects of turbid chaenomeles juice on fermentation and to ensure the sensory quality of fruit wine, pectinase was used to clarify chaenomeles juice in this experiment. The gradient of pectinase dosage was designed into 20, 25, 30, 35, 40 and 45 mg/L. The optimum dosage of pectinase selected was used for clarifying chaenomeles juice. chemical clarification method (bentonite and chitosan) was employed to clarify and stabilize the raw wine, improve its appearance and reduce the risk of diseases in the storage process, by designing the gradient of bentonite dosage, 0.8, 0.9, 1.0, 1.1, 1.2 and 1.3 g/L to select the most suitable amount of bentonite, whereas the gradient of chitosan for 1.2, 1.5, 1.8, 2.1, 2.4 and 2.7 g/L to the same effect. The optimum dosage of bentonite and chitosan were applied to chaenomeles wine to analyze and compare the clarifying effect of both two to wine, while the clarification indexes, such as anthocyanin content, turbidity, chroma and light transmittance were determined. The best dosage of pectinase was selected as 35 mg/L, the color 2.425, the light transmittance 54.7% and the best clarification effect of chaenomeles juice was obtained after clarifying 5 h. The best clarifying effect was obtained under the light permeability 29.3% and turbidity 90.04 NTU in chaenomeles wine through the best bentonite dosage of 1.0 g/L after clarifiing 12 h. While the best dosage of chitosan to clarify the chaenomeles wine was 1.8 g/L, which made the light permeability 9.80% and the turbidity 199.2 NTU after 12 h. The chaenomeles juice treated with 35 mg/L pectinase had higher the clarification efficiency and effect, and the color remained intact. While the clarifying effect of 1.0 g/L bentonite solution on chaenomeles liquor was better than that of 1.8 g/L chitosan treatment.

INTRODUCTION

Chaenomeles juice contains colloidal substances including pectin, gum and protein, small pulp particles, dissolving substances with colloidal, molecular or ionic characters, and substance suspending rinds, seeds and cellulose, which are potentially dangerous to later fermentation, and impregnated fermentation will affect adversely the aroma of chaenomeles wine.

The protein, pectin and polyphenols substance coexisting fruit wine for a long time would produce turbid colloid and precipitate [1]. The new wine contained suspended yeast, bacteria, condensed proteins, tannins, mucus and fragments of berry tissue. The residual microorganism metabolized the composition of alcohol and damaged the colloidal balance of the wine, causing fog, turbidity or precipitation, which might harm the storage of fruit wine. Some data showed that the clarification methods included centrifugal separation and diatomite plate filter, and more clarifiers used were chitosan, gelatin and bentonite [2]. Taking into account the higher clarifying cost of centrifugal separation and difficult control to operative technique, support plate of diatomaceous plate filter needs to be replaced regularly

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and higher cost, waste diatomaceous soil needed be removed after the clarifications with the larger labor intensity, the board exposed to the board caused easily the surface mold or leakage, and more wine head and tail appeared, therefore, the way adding clarifying agent was selected. In order not to affect the late fermentation, the clarification of fruit juice usually adopted cold treatment clarification method and pectinase clarification method. For the clarifications of fruit wine, chemical clarifications could be added to achieve the purpose.

The clarifying agents in this experiment were pectinase, saponin and chitosan. The pectinase, the appearance of light yellow powder, obtained from aspergillus niger by fermentation, which mainly used for juicing and clarification of fruit and vegetable juice drinks and wine with good effect on decomposing pectin. The stable range of pectinase pH was 3.0 - 6.0, the optimal value of 3.5 - 5.5, the enzyme activity decreased rapidly at 60 °C, and the optimum temperature was 45-50 °C [3].

The enzyme preparation added in chaenomeles juice could hydrolyze the pectin, and make the other gels in chaenomeles generate copolymerization by losing the protective effect of pectin. Bentonite was an inorganic mineral gel refined by natural bentonite. The bentonite would inflate and disperse in the water when being immersed in hot water, form uniform colloidal suspension through the dispersion with negative charge. While most of the turbid substance with positive charge in wine would be attracted by bentonite with the negative charge, which caused flocculation promoting wine clarify [4-5].

Chitosan also known as deacetylchitin, which widely existing in nature by the chitin deacetylation, the chemical name is polyglucosamine (1-4) - 2 - amino - B - D glucose that a white or off-white translucent flake or powder solid, and it is tasteless, odorless and non-toxic. Chitosan as a chemical clarifier has been widely used in the clarification process due to its good clarification effect. The results showed that chitosan could promote the separation of solid and liquid during the clarification process of the juice, so that the light transmittance of the juice increased significantly. When the amount of chitosan is not enough to take charge neutralization and adsorption bridging action with protein and tannic acid in wine, the colloidal particles are difficult to flocculate and precipitate. Fruit juice or wine contains a large number of negative charge pectin, cellulose, tannin and polypentose and other substances, will make the fruit juice turbid during storage. When the positive charge of chitosan and the above negative charge substance adsorbed and flocculated, the treated clarified juice or wine will become a stable thermodynamic system, so it can be stored for a long time without turbidity. Chitosan as a safe and non-toxic natural clarifier interacts with negatively charged colloid, which has excellent flocculation performance, plays a certain role in proteins, pectin and polyphenols, and it is characterized by rapid, simple and easy operation. The flavor and nutrition of the product were not affected, as well as the biological stability was good [6-9].

MATERIALS AND METHODS

Materials

Chaenomeles was from Yunxian county, Lincang city, Yunnan; Chaenomeles juice was squeezed by the above chaenomeles, Wine Technology Laboratory, Chemistry and Life Science College, Chuxiong Normal University; Chaenomeles wine was made in the above Chaenomeles juice.

Technological process: Raw materials - sorting - peeling and coring - crushing-clarifying - deacidifying - adding sugar - alcoholic fermentation - malolactic fermentation - clarifying - stability test - bottling - storage.

Preparation and Adding of Clarifier

Bentonite Solution

Bentonite, chitosan and pectinase are all food grades.

Preparation of 100 g/L Bentonite solution: 10 g Bentonite dissolved in 50 °C hot water was constant volume to 100 mL with distilled water, then stirred well to avoid caking. After soaking for 24 h to full swell, the solution was mixed evenly for standby application. The solution was stirred thoroughly before adding into the liquor, and wine lees was separated after 12 hours.

Adding method of 100 g/L bentonite solution was as follows: (1) 0.8 g/L: 0.40 mL of bentonite solution prepared was added into 50 mL chaenomeles wine sample; (2) 0.9 g/L: 0.45 mL of bentonite solution prepared was added into 50 mL chaenomeles wine sample; (3) 1.0 g/L: 0.50 mL of bentonite solution prepared was added into 50 mL chaenomeles wine sample; (4) 1.1 g/L: 0.55 mL of bentonite solution prepared was added into 50 mL chaenomeles

wine sample; (5) 1.2 g/L: 0.60 mL of bentonite solution prepared was added into 50 mL chaenomeles wine sample; (6) 1.3 g/L: 0.65 mL of bentonite solution prepared was added into 50 mL chaenomeles wine sample.

Chitosan Solution

Preparation of chitosan solution: The methods referred to Tran et al. [10], with some modifications. After 0.05 g citric acid, added into 8.95 mL deionized water, was dissolved in heat condition, 1 g chitosan with 96% deacetylation degree was added with stirring for more than 10 hours of soak, which was 100 g/L of chitosan solution.

Adding method of chitosan solution was as follows: (1)1.2 g/L: 0.60 mL chitosan solution prepared was added into 50 mL chaenomeles wine sample; (2)1.5 g/L: 0.75 mL chitosan solution prepared was added into 50 mL chaenomeles wine sample; (3)1.8 g/L: 0.90 mL chitosan solution prepared was added into 50 mL chaenomeles wine sample; (4)2.1 g/L: 1.05 mL chitosan solution prepared was added into 50 mL chaenomeles wine sample; (5)2.4 g/L: 1.20 mL chitosan solution prepared was added into 50 mL chaenomeles wine sample; (6)2.7 g/L: 1.35 mL chitosan solution prepared was added into 50 mL chaenomeles wine sample.

Enzymatic Clarification

The method was employed mainly in clarification of chaenomeles juice. (1) 6-50 mL colorimetric tubes were added 50 mL chaenomeles juice, respectively, then added 20, 25, 30, 35, 40 and 45 mg/L pectinase in proportion, respectively; (2) Pectinase was dissolved and hydrolyzed at room temperature with slowly stirred glass rod; (3) The supernatant was used to measure the chromaticity and light transmittance after 5 h, and the light transmittance was used as the main index to determine the amount of pectinase. (4) The optimal amount of pectinase was selected for the experiment. After 12 hours of treatment, the turbidity, chromaticity, light transmittance and anthocyanin content of chaenomeles juice were determined.

Bentonite Clarification

The method was employed mainly in clarification of chaenomeles wine. (1) 100 g/L bentonite solution was prepared; (2) 6 colorimetric tubes were added 50 mL chaenomeles wine, respectively; (3) After the bentonite solution mixed evenly were added to the chaenomeles wine sample successively according to the set gradient, 0.8 g/L, 0.9 g/L, 1.0 g/L, 1.1 g/L, 1.2 g/L and 1.3 g/L, and then mixed. (4) After standing at room temperature for 12 h, the turbidity and light transmittance were measured, and the optimal amount of bentonite was selected according to the values of turbidity and light transmittance. (5) The turbidity, chromaticity, transmittance and anthocyanin content of chaenomeles wine were determined by using the selected amount of bentonite.

Chitosan Clarification

The method was employed mainly in Clarification of chaenomeles wine. (1) 100 g/L Chitosan solution was prepared; (2) Six colorimetric tubes were added 50 mL chaenomeles wine, respectively; (3) Chitosan solution was added into chaenomeles wine at the concentration gradient of 1.2 g/L, 1.5 g/L, 1.8 g/L, 2.1 g/L, 2.4 g/L and 2.7 g/L respectively. Adding chitosan while stirring to make the chitosan fully contact with the particles in the solution; (4) After 12 h at room temperature, various clarification indexes were determined, and the optimal amount of chitosan in the clarification process of chaenomeles wine was analyzed; (5) The selected optimal amount of chitosan was used to this experiment with measure of the turbidity, chromaticity, light transmittance and anthocyanin content of chaenomeles juice; (6) The clarification effect of bentonite and chitosan on chaenomeles wine was compared and analyzed.

Determination of Chromaticity

(1) The pH value of the sample solution was determined with a pH meter.
(2) Preparation of buffer solution
Buffer solution A: 0.2 mol/L disodium hydrogen phosphate.
7.12 g disodium hydrogen phosphate weighed was kept in a 200 mL of constant volume.
Buffer solution B: 0.2 mol/L citric acid.

4.2 g citric acid weighed was in a 200 mL of constant volume.

The mixture pH of buffer solution A and B was determined directly by pH meter before preparing buffer solution, so that the buffer solution's pH value was the same as the sample solution's pH value.

(3) 2 mL sample solution was absorbed through a pipette into a 25 mL colorimetric tube before diluted to 25 mL with a buffer of the same pH value as the sample solution. The sample was placed to the colorimetric tank for 15 minutes. Bubbles observed in the colorimetric dish were droved away before starting colorimetry. Absorbance was measured with 1 cm cuvette at λ_{420}, λ_{520} and λ_{620}, with distilled water as the blank.

(4) Calculation:

$$Chroma = (A_{420} + A_{520} + A_{620}) \times dilution\vec{}ratio$$

The result was kept as a decimal place.

Determination of Anthocyanin Content

(1) Preparation of buffer

PH1.0 buffer solution: 0.93 g of potassium chloride was weighed accurately by an electronic analytical balance before added to about 480 mL by distilled water, and then distilled water was used to fix volume to 500 mL after adjusting to pH1.0 by hydrochloric acid and acidity meter.

PH4.5 buffer solution: 16.405 g anhydrous sodium acetate was weighed accurately by an electronic analytical balance before added to about 480 mL by distilled water, and then distilled water was used to fix volume to 500 mL after adjusting to pH1.0 by hydrochloric acid and acidity meter.

(2) Extraction of anthocyanin

The anthocyanin extract was obtained by 1.0 mL of the sample adding 70% ethanol extract at a ratio of 1:30 (g/mL), and then extracted under ultrasonic for 25 min before filtration.

(3) Determination of anthocyanin content

PH-differential method: 1 mL sample solution was diluted to 10 mL with buffer solution of pH 1.0 and 4.5, and placed in dark place for 15 minutes of balance, respectively. The absorbance was measured at 510 nm and 700 nm of the visible spectrophotometer on the cuvette with a diameter of 1 cm. Distilled water was used as blank control. The anthocyanin content was calculated by the following formula:

$$A = [(A_{510} - A_{700})pH_{1.0} - (A_{510} - A_{700})pH_{4.5}]$$

Anthocyanin concentration:

$$\mathrm{C}\ (mg/L) = \frac{A \times MW \times DF \times 1000}{\varepsilon \times l}$$

Where: $(A_{510}\text{-}A_{700})$ pH1.0 — the difference value of the absorbance at the wavelenth of 510 nm and 700 nm which added sample solution with pH 1.0 buffer solution;

$(A_{510}\text{-}A_{700})$ pH 4.5 — the difference value of the absorbance at the wavelenth of 510 nm and 700 nm which added sample solution with pH 4.5 buffer solution;

MW=449.2 — molecular weight of cornflower - 3 - glucoside, mg/mol;

DF—Dilution multiple of sample solution;

ε=26900 — the molar extinction coefficient of cornflower - 3 - glucoside, mol^{-1};

l - Diameter of light path of the cuvette, 1 cm.

Measurement of Light Transmittance

All the light transmittance values in this experiment were measured at the wavelength of 700 nm on UV-5500 visible spectrophotometer.

Statistical Analysis

SPSS software was used for all statistical analysis. All factors were analyzed by standard deviation.

RESULTS AND DISSCUSS

Treatment of Papaya Juice with Pectinase

Selection of Optimum Pectinase Dosage

After papaya was squeezed into juice in this experiment, part of which was taken out and clarified by adding pectinase in gradient, meanwhile chromaticity and light transmittance were measured respectively to select the optimal amount of pectinase. As shown in Table 1, the light transmittance increased gradually with the increase of the amount of pectinase in the experiment clarifying papaya juice with pectinase. When the amount of pectinase was up to 35 mg/L, the light transmittance reached the maximum. After that, when the amount of pectinase was increased continually, the light transmittance began to decrease gradually.

The conclusion obtained in the determination of chromaticity was that the chromaticity decreased gradually with the increase of the amount of pectinase. When the value of pectinase was raised to 35 mg/L, the chromaticity was reduced to the minimum value. Along with further augment as far as the amount of pectinase, the chromaticity began to gradually increase again. One conclusion can be drawn from the above two groups of datum: the optimal amount of pectinase was 35 mg/L in the papaya juice clarification experiment.

As could be seen from this experiment, when papaya juice was treated with 35 mg/L and 40 mg/L of pectinase, respectively, the difference in light transmittance was small, only by 0.6%, however the difference in chromaticity was 1.28. Therefore, if the clarification effect of papaya juice wanted to be improved, 35 mg/L of pectinase should be selected as thought from economical and economical view. Of course, 40 mg/L of pectinase should be the best option for treatment if high chromaticity was to be retained.

TABLE 1. Screen of the optimum dosage of pectinase

Pectinase dosage (mg/L)	Light transmittance (%)	Chroma
0	0.92±0.01	8.14±0.26
20	5.02±0.13	5.99±0.23
25	45.30±1.21	3.25±0.17
30	30.10±1.32	2.48±0.25
35	54.70±1.12	2.43±0.36
40	54.10±1.35	3.71±0.29
45	14.50±1.24	4.89±0.34

Treatment Effect of Pectinase

As shown in Table 2, the anthocyanin concentration in papaya juice was decreasing with the prolonged treatment time of pectinase. After 6 hours of treatment, the anthocyanin concentration was decreased to 0 mg/L. The light transmittance increased with the prolonging of pectinase treatment time in the light transmittance test. The turbidity of papaya juice decreased from the initial over 1000 NTU to 245 NTU, and reduced to 144.6 NTU again after 6 hours. Whereas the chromaticity decreased from the original 26.75 to 2.65 obviously, but there was still some reservation. Therefore, clarify papaya juice through pectinase has the advantages, such as short time, high efficiency and good effect, which has great effect on the deposition of suspended matter in papaya juice. Pectinase was helpful to extract the natural components in the suspension of papaya pulp, which was of great benefit to the color and aroma of extraction and retention in papaya wine.

TABLE 2. The effect of treatment with pectinase to papaya wine

	Clarity Index			
Handling time (h)	Anthocyanin content (mg/L)	Light transmittance (%)	Turbidity (NTU)	Chroma
0	0.72±0.13	0.36±0.25	1675.2±2.59	26.75±0.29
3	0.05±0.01	21.10±1.12	245.3±3.25	6.00±0.31
6	0	35.70±1.32	144.6±1.26	2.65±0.12

Treatment of Papaya Wine with Bentonite

Determination of Bentonite Dosage

When the amount of bentonite was appropriate, the clarified wine would emerge obviously fresher and more harmonious taste, which was conducive to the formation of the pawpaw wine vinosity [11]. As shown in Table 3, with the increase of bentonite content, the light transmittance of pawpaw wine went up gradually. When the amount of bentonite reached 1.0 g/L, the light transmittance of pawpaw wine reached the maximum value. After that, the amount of bentonite was continually increased, the light transmittance decreased gradually with a slow rate. The main reason giving rise to this phenomenon was obvious that the protein occurred flocculated and precipitated reaction with bentonite in papaya wine, and more bentonite content had little effect on the clarification of papaya wine, even led to turbidity [12-13]. The turbidity of papaya wine displayed decreased trend before increase with the augment of the amount of bentonite. When the amount of bentonite was 1.0 g/L, the turbidity reached the maximum. It could be concluded from the above analysis that the optimal dosage clarifying papaya wine was 1.0 g/L bentonite. Bentonite would form colloidal suspension after expansion by absorbing water. These colloidal particles were negatively charged, while the turbid substance such as proteins in papaya wine was mostly positively charged. Therefore, the cloudy substance and bentonite formed a flocculent precipitate due to the mutual attraction of positive and negative charges after adding bentonite, so that the pawpaw wine could be clarified, which relied on the ability of bentonite to absorb proteins, and the electrical charge of protein molecules, with a positive charge, determined by the wine pH [14].

TABLE 3. Determination of bentonite dosage

Dosage of bentonite (g/L)	Light transmittance (%)	Turbidity (NTU)
0.8	19.1±1.12	118.80±2.32
0.9	21.9±1.34	112.20±4.26
1.0	29.3±1.26	90.04±1.18
1.1	28.1±1.46	92.80±1.25
1.2	27.8±1.25	100.00±2.31
1.3	23.8±1.46	121.70±1.49

Clarifying Effect of Bentonite

Usually, the stability of anthocyanin was affected by many factors, such as pH value, temperature, additives and light [15]. Therefore, content of anthocyanin in papaya wine was very small and unstable. After 12 h of bentonite clarification, the content of anthocyanin had been reduced from 0.08 to 0 mg/L (Table 4). In the clarification experiment of papaya juice at the early stage of fermentation, the anthocyanin concentration had been reduced to 0 mg/L, therefore a small amount of anthocyanin during the brewing process stemmed mainly from saccharification liquid of corn starch added to papaya juice by adjusting sugar method [16-17]. Bentonite had an excellent clarification effect on papaya wine. After treatment with 1.0 g/L bentonite, the light transmittance of papaya wine increased from 9.12% to 54.90%, the turbidity decreased from 367.90 NTU to 49.23 NTU, and the chroma decreased from 7.00 to 1.99, respectively.

TABLE 4. The clarification effect of bentonite on papaya wine

	Anthocyanin (mg/L)	Light transmittance (%)	Turbidity (NTU)	Chroma
Before clarification	0.08±0.01	9.12±0.12	367.90±3.46	7.00±0.18
After clarification	0	54.90±1.11	49.23±1.28	1.99±0.08

Effect of Chitosan to Papaya Wine

Determination of the Optimum Chitosan Dosage

As shown in Table 5, the papaya wine was clarified with chitosan solution. Within the range of 1.2-2.7 g/L, the experiment was conducted with 0.3 g/L as the gradient. The results showed that after the papaya wine was treated with different concentrations of chitosan solutions for 12 hours, the light transmittance increased gradually with the increase of chitosan dosage. When the chitosan dosage reached 1.8 g/L, the light transmittance reached also the maximum. After that, when chitosan dosage was continually increased, the light transmittance began to decrease gradually. While the turbidity of papaya wine decreased gradually with the increase of chitosan dosage. When the chitosan dosage was 1.8g/L, the turbidity drop down to the minimum level and then increased again with the increase of chitosan dosage. Therefore, the optimum dosage of chitosan in clarification experiment of papaya wine was identified as 1.8g/L. In the clarification experiment of papaya wine, the charge neutralization and adsorption bridging action of chitosan increased with the addition of flocculant, and its clarification effect would be better and better. However, excessive addition of chitosan would lead to the encapsulation of all the particles in the liquor, which led to loss of the bridging effect between the particles. At the same time, secondary adsorption would occur on the surface of the colloid, which made the particles in a stable state without advantage to clarification. In addition, too much chitosan would increase the viscosity and turbidity of the liquor, so that the adsorption of chitosan to the effective components of the liquor would also increase [18], which was easy to cause adverse effects on the flavor of papaya wine.

TABLE 5. Select of optimum chitosan dosage

Chitosan dosage (g/L)	Light transmittance (%)	Turbidity (NTU)
1.2	1.68±0.01	931.1±3.45
1.5	5.76±0.25	328.4±2.26
1.8	9.80±0.34	199.2±1.18
2.1	1.59±0.15	911.8±5.26
2.4	1.55±0.08	923.0±2.15
2.7	1.54±0.06	949.2±1.38

The Effect of Chitosan on Clarification of Papaya Wine

In the clarification test, chitosan, as a clarifying agent, had the advantages, such as short clarification time, simple operation, low cost and good clarification effect [19-20]. The indicators of the papaya wine, treated with 1.8 g/L chitosan solution, were determined after 12 hours of the clarification. As shown in table 6, chitoans had a significant effect on clarifying papaya wine. The content of anthocyanin decreased from 0.080mg /L before clarification to 0.07 mg/L now, and the turbidity decreased also from 367.90 NTU to 83.56 NTU. However, the chromaticity, remained relatively good, was decreased only from 7.00 to 5.04. On the contrary, the light transmittance increased significantly from 9.12% to 34.00%. When adding chitosan, with the clarification effect, into papaya wine, the wine color could be changed from the initial pale yellow to dark yellow because of the affinity of chitosan and polyphenols, which improved greatly the appearance quality of the papaya wine [21-23]. Similar results were obtained in this experiment.

TABLE 6. The effect of clarification with chitosan

	Anthocyanin content (mg/L)	Light transmittance (%)	Turbidity (NTU)	Chroma
Before clarification	0.08±0.02	9.12±0.26	367.90±3.28	7.00±0.28
After clarification	0.07±0.01	34.00±0.38	83.56±1.16	5.04±0.36

CONCLUSIONS

In the clarification experiment of papaya juice, the 54.70% transmittance and 2.43 chroma was obtained by 35 mg/L pectinase treatment to the papaya juice, while the 54.10% transmittance and 3.71 chroma was obtained by 40 mg/L pectinase treatment to the papaya juice. The transmittance difference under two pectinases of treatment

concentrations was only 0.6%, whereas the chroma difference under two concentrations was 1.28. Therefore, 35 mg/L pectinase should be selected in order to improve the clarity of papaya juice, nevertheless 40 mg/L pectinase should be the best option if higher chroma wanted to be required.

The best bentonite dosage, 1.0 g/L, and the optimal chitosan dosage, 1.8 g/L, was selected by setting the concentration gradient of clarifier and analyzing the turbidity and light transmittance of papaya wine before and after treatmen.

The optimal dosage of bentonite and chitosan were added to the papaya wine, respectively. After 12 h, the two samples of the turbidity, chromaticity, transmittance and anthocyanin content were determined. The transmittance of papaya wine ascended by from 9.12% before treatment to 54.9% after bentonite treatment and only 34.0% after chitosan treatment. The turbidity of papaya wine decreased from 367.9 NTU before treatment to 49.23 NTU after bentonite treatment, and to 83.56 NTU after chitosan treatment. The chroma of papaya wine decreased from 7.00 before treatment to 1.99 after bentonite treatment and to 5.04 after chitosan treatment. While the anthocyanin of papaya wine decreased from 0.08 mg/L before treatment to 0 mg/L after bentonite treatment, and to 0.07 mg/L after chitosan treatment. It could be concluded that bentonite was obviously better than chitosan in clarifying the same batch of papaya wine in the same period of time. However, chitosan could retain the color and anthocyanin content of papaya wine.

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REFERENCES

1. O. S. Lukanin, S. M. Gunko, M. T. Bryk and R. R. Nigmatullin, J. Food Eng. **60**, 275-280 (2003).
2. A. Mierczynska-Vasilev and P. A. Smith, Aus. J. Grape Wine R. **21,** 615-626 (2015).
3. J. C. Lin, L. H. Zhu, Z. P. Wan, Z. M. Huang and G. Q. Chen, Food Sci. **12,** 074 (2006).
4. S. C. Van Sluyter, J. M. McRae, R. J. Falconer, P. A. Smith, A. Bacic, E. J. Waters and M. Marangon, J. Agr. Food Chem. **63,** 4020-4030 (2015).
5. K. Jeyaram, "Studies on the selection of suitable yeast cultures and standardization of fermentation conditions for wine making from Banana, Papaya and Grapes" Ph.D. thesis, Tamil Nadu Agricultural University, 2002.
6. X. Zhang, H. Wu and C. Wang, Transactions of the Chinese Society of Agricultural Engineering, **29,** 267-276 (2013).
7. M. Rinaudo, Prog. Polym. Sci. **31,** 603-632 (2006).
8. V. Rungsardthong, N. Wongvuttanakul, N. Kongpien and P. Chotiwaranon, Process Biochem. **41,** 589-593 (2006).
9. J. Oszmianski, M. Wolniak, A. Wojdylo and I. Wawer, J. Sci. Food Agr. **87,** 573-579 (2007).
10. L. Tran, S. H. Ching and C. U.S. Turner, Patent Application No. 14/648,292 (2015).
11. A. L. Fudge, M. Schiettecatte, R. Ristic, Y. Hayasaka and K. L. Wilkinson, Aust. J. Grape Wine R. **18,** 302-307 (2012).
12. M. Esti, I. Benucci, C. Lombardelli, K. Liburdi and A. M. V. Garzillo, Food Bioprod. Process. **91,** 595-598 (2013).
13. I. Benucci, C. Lombardelli, K. Liburdi, G. Acciaro, M. Zappino and M. Esti, J. Food Sci. Tech. **53,** 1130-1139 (2016).
14. H. Mine Kurtbay, Z. Bekçi, M. Merdivan and K. Yurdakoç, J. Agr. Food Chem. **56,** 2541-2545 (2008).
15. A. Patras, N. P. Brunton, C. O'Donnell and B. K. Tiwari, Trends Food Sci. Tech. **21**, 3-11 (2010).
16. C. L. Luchese, N. Sperotto, J. C. Spada and I. C. Tessaro, Int. J. Biol. Macromol. **104**, 11-18 (2017).

17. D. Lemlioglu-Austin, N. D. Turner, C. M. McDonough and L. W. Rooney, Molecules, **17**, 11124-11138 (2012).
18. Y. Xu, S. Purton and F. Baganz, Bioresource Technol. **129**, 296-301 (2013).
19. O. Tastan and T. Baysal, Food Chem. **180**, 211-218 (2015).
20. L. T. Cesar, M. de Freitas Cabral, G. A. Maia, R. W. de Figueiredo, M. R. A. de Miranda, P. H. M. de Sousa, I. M. Brasil and C. L. Gomes, J. Food Sci. Tech. **51**, 3293-3300 (2014).
21. M. Cerreti, K. Liburdi, I. Benucci, S. E. Spinelli, C. Lombardelli and M. Esti, LWT-Food Sci. Technol. **82**, 58-65 (2017).
22. L. Zhang, "The effects of high pressure processing, browning additives, and storage period on the inactivation of polyphenol oxidase in nine varieties of pawpaw (Asimina Triloba L.) pulp" MS thesis, Ohio University, 2016.
23. L. Sánchez-González, C. Pastor, M. Vargas, A. Chiralt, C. González-Martínez and M. Cháfer, Postharvest Biol. Tec. **60**, 57-63 (2011).