

The Variation Of Methanol Content In The Process Of Clarifying Honeydew Juice With Water-soluble Chitosan

Mingrui Wang^{1*}, Zeyu Ping¹, and Xin Li^{1,2}

¹ Kaifeng University, Kaifeng 475004, China

² Kaifeng Fermented Food Engineering Technology Research Center, Kaifeng 475004, China

* Corresponding author. E-mail: kfdx202511@163.com

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Objective: In the process of clarifying Honeydew juice with chitosan, the change of methanol content in Honeydew juice was mainly investigated. **Methods:** The effects of chitosan addition amount, initial pH value of clarification, clarification time and clarification temperature on the clarification effect and methanol production of Honeydew juice were investigated by single factor and orthogonal test with the light transmittance and methanol production as indicators. **Results:** The order of influencing factors on methanol production of honeydew juice after clarification was as follows: initial pH>temperature>chitosan addition>clarification time. The minimum amount of methanol production of Honeydew juice and the best clarification conditions were 0.04 g/100 mL chitosan addition, pH=5.5, temperature 35 °C, clarification for 30 minutes. Under this process condition, the methanol content is 28.6 mg/L, and the light transmittance can reach 96.35%. **Conclusion:** Chitosan can significantly reduce the methanol content in Honeydew juice.

Keywords: Chitosan, Pectinase; Clarification; Methanol content

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1. introduction

Fruit wines have a relatively high content of pectin in their production raw materials, which may lead to excessive methanol content during the production process. Therefore, in China's standards for distilled spirits and their blended liquors, there are limits on methanol content [1]: white wine $\leq 250\text{mg/L}$, red wine $\leq 400\text{mg/L}$. However, there are no clear limit standards for methanol content in fermented wines brewed from other fruits. A large number of studies have shown that the methanol content in fermented wines produced from common fruits such as fragrant pears, grapefruits, hawthorns, and lychees easily exceeds the methanol limit standard for wine, which restricts the production of fruit fermented wines [2, 3]. When pectin and protein are removed through juice clarification, the methanol content can be reduced. The clarification process is a crucial link in the processing of clear juice. Currently,

enzyme clarification, ultrafiltration clarification, and clarification using clarifying agents (such as gelatin, bentonite, and polyvinylpyrrolidone (PVPP)) are widely used in the process [4–6]. At present, there is a large amount of research data on the selection of clarification process conditions [7–10], but there are few studies on the methanol content in juice after clarification.

Some studies have used chitosan as a clarifying agent [11, 12]. Chitosan is biodegradable, biocompatible, safe, non-toxic, and edible, and has been widely used as an emulsifier, stabilizer, and thickener in the food industry. Water-soluble chitosan contains amino and hydroxyl active groups, and has amphoteric properties. It can interact with negatively charged proteins, cellulose, and pectin in fruit pulp to produce flocculation. In this experiment, chitosan was used as a clarifying agent to treat Honeydew juice to reduce the pectin content. Pectinase was used to treat soluble pectin, and gas chromatography was used to

accurately determine the content of methanol produced in the subsequent process. Thus, the impact of chitosan clarification process conditions on the methanol content of Honeydew juice was analyzed; the chitosan clarification process conditions for honeydew juice were determined to achieve the goal of reducing the methanol content in the system, providing technical support for the processing of melon fruits.

2. materials and methods

2.1. Materials, instruments, and reagents

2.1.1. Materials

Honeydewmelons (Lankao honeydew, provided by Lankao County Agricultural Cooperative): uniform in size, fresh in quality, and moderately mature.

2.1.2. Instruments and equipment

- Gas chromatograph (FID detector): Fuli Analytical Instrument Co., Ltd.
- UV-1800PC UV-Vis spectrophotometer: Shanghai Mapada Instruments Co., Ltd.
- Electronic analytical balance: Ohaus Corporation (USA)
- TG16-WS centrifuge: Shanghai Luxiangyi Centrifuge Instrument Co., Ltd.
- JE-B05B juicer: Yuyao Canhong E-Commerce Co., Ltd.
- HH-3 digital display constant temperature water bath: Yuyao Mingwei Instrument Factory
- PHS-3C precision pH meter: Shanghai Dapu Instrument Co., Ltd.
- Digital display refractometer (sugar content meter): Dongguan Donglaida Electronics Co., Ltd.

2.1.3. Main reagents

The chemical reagents and enzymes used in this work are listed below:

- Pectinase (50,000 U/g, Ningxia Heshibi Biotechnology Co., Ltd.)
- Cellulase (50,000 U/g, Ningxia Heshibi Biotechnology Co., Ltd.)
- Chitosan (AR grade, degree of deacetylation > 95%, Shanghai Jingchun Reagent Co., Ltd.)
- Galacturonic acid (purity > 99.9%, Shanghai Ruji Biotechnology Development Co., Ltd.)

- Glucose, citric acid, ascorbic acid, methanol standard solution, 2,6-dichlorophenol indophenol, and 3,5-dinitrosalicylic acid: all of analytical grade.

2.2. Experimental Methods

2.2.1. Preparation and treatment process of honeydew juice

(1) Preparation of honeydew juice

Honeydew → Cleaning → Peeling and seeding → Dicing → Juicing → Coarse filtration → Honeydew juice

(2) Treatment process of honeydew juice

Transfer 100 mL of fresh honeydew juice into a 250 mL conical flask, adjust the pH with citric acid, add an appropriate amount of chitosan, and mix thoroughly. Heat the mixture in a constant temperature water bath at a specific temperature for a certain clarification time, then centrifuge at 4800r/min for 10 minutes. After centrifugation, take 10 mL of the supernatant to determine the light transmittance (T%) at 680 nm. Add 0.04 g of pectinase to the remaining supernatant, perform enzymatic hydrolysis in a water bath at 35°C for 2 hours, followed by distillation separation. The methanol content in the distilled solution was determined by gas chromatography.

The change in methanol content in the final distilled solution before and after adding chitosan to the fresh honeydew juice system was compared to confirm the clarification effect of chitosan [13, 14].

2.2.2. Solution preparation

(1) 2% Chitosan solution

Accurately weigh 2.00 g of chitosan powder, dissolve it in approximately 50 mL of distilled water with stirring until fully dissolved, and dilute to 100 mL in a volumetric flask for later use.

(2) 2% Pectinase solution

Accurately weigh 5.00 g of pectinase, dissolve it in approximately 100 mL of distilled water with stirring until fully dissolved, and dilute to 250 mL in a volumetric flask with distilled water for later use.

(3) 10% Cellulase solution

Accurately weigh 10.00 g of cellulase, dissolve it in approximately 30 mL of distilled water with stirring until fully dissolved, and dilute to 100 mL in a volumetric flask for later use.

2.2.3. Determination methods

(1) Determination of clarity by light transmittance

Take 10 mL of the clarified supernatant of the treated honeydew juice after centrifugation, transfer it to a 1 cm cuvette, use pure water as the reference, and determine the light transmittance of the solution at 680 nm with a UV-Vis spec-

trophotometer. The clarity is expressed as T (%).

(2) Determination of methanol content

Determine the methanol content in accordance with GB 5009.266-2016 National Food Safety Standard - Determination of Methanol in Foods. Distill the solution after pectinase hydrolysis, then use a capillary chromatographic column (GC-FID) to determine the methanol content in the solution.

(3) Quantitative determination of pectin

Determine the pectin content by spectrophotometry in accordance with the method specified in NY/T 2016-2011 Determination of Pectin Content in Fruits and Their Products. Precipitate the pectin in the sample with absolute ethanol; the pectin generates galacturonic acid after hydrolysis. Galacturonic acid undergoes a condensation reaction with the carbazole reagent in concentrated sulfuric acid to form a fuchsin compound. Use a UV-Vis spectrophotometer to determine the absorbance at 525 nm, draw a standard curve using galacturonic acid as the standard substance, and conduct quantitative analysis of the pectin content in the solution.

(4) Determination of total sugar

In accordance with NY/T 2332-2013 Determination of Total Sugar Content in Red Ginseng - Spectrophotometric Method, after the carbohydrates in the sample are hydrolyzed with hydrochloric acid, water-soluble sugars and water-insoluble sugars are converted into reducing sugars. When the reducing sugars are heated together with 3,5-dinitrosalicylic acid, the nitro compound is reduced to an amino compound (brownish-red). Determine the content of reducing sugars by spectrophotometry, and then convert it to the total sugar content.

(5) Determination of total acidity

Determine the total acidity in accordance with GB 12456-2021 National Food Safety Standard - Determination of Total Acidity in Foods. The second method, pH meter potentiometric titration, is adopted, and the total acidity is expressed as citric acid.

(6) Determination of pH value

Determine the pH value of the solution with a PHS-3C precision pH meter in accordance with GB 5009.237-2016 National Food Safety Standard - Determination of pH Value in Foods.

(7) Determination of vitamin C content

Determine the ascorbic acid (vitamin C) content in the sample by the third method, 2,6-dichloroindophenol titration, specified in GB 5009.86-2016 National Food Safety Standard - Determination of Ascorbic Acid in Foods.

2.3. Single-Factor experiments on chitosan clarification

2.3.1. Effect of chitosan addition amount on light transmittance and methanol content

Take 100 mL of fresh honeydew juice each and place them in 8 conical flasks (250 mL). Adjust the pH to 5.5 using 20% citric acid, then add chitosan at concentrations of 0.00, 0.02, 0.04, 0.06, 0.08, 0.10, 0.12, and 0.16 g per 100 mL of fresh honeydew juice, mixing thoroughly. Clarify the mixture in a 35°C water bath for 30 minutes, then sample and centrifuge to determine the light transmittance (T%). Add 0.2 mL of pectinase and 2 mL of cellulase to the remaining supernatant, perform enzymatic hydrolysis in a 35°C water bath for 2 hours, followed by distillation. Determine the methanol content (mg/L) using gas chromatography.

2.3.2. Effect of clarification temperature on light transmittance and methanol content

Take 100 mL of fresh honeydew juice each and place them in 8 conical flasks (250 mL). Adjust the pH to 5.5 using 20% citric acid, then add chitosan at a concentration of 0.04 g per 100 mL of juice. Clarify the mixtures in water baths at controlled temperatures of 20, 25, 30, 35, 40, 45, and 50°C respectively for 30 minutes. Sample and centrifuge to obtain the clarified liquid, then determine its light transmittance (T%). Add 0.2 mL of pectinase and 2 mL of cellulase to the remaining supernatant, perform enzymatic hydrolysis in a 35°C water bath for 2 hours, followed by distillation. Determine the methanol content (mg/L) using gas chromatography.

2.3.3. Effect of initial clarification pH on light transmittance and methanol content

Take 100 mL of fresh honeydew juice each and place them in 6 conical flasks (250 mL). Adjust the pH to 4.0, 4.5, 5.0, 5.5, 6.0, and 6.45 (initial pH of the honeydew juice system) respectively using 20% citric acid, then add chitosan at a concentration of 0.04 g per 100 mL of juice. Clarify the mixtures in a 35°C water bath for 30 minutes. Sample and centrifuge to obtain the clarified liquid, then determine its light transmittance (T%). Add 0.2 mL of pectinase and 2 mL of cellulase to the remaining supernatant, perform enzymatic hydrolysis in a 35°C water bath for 2 hours, followed by distillation. Determine the methanol content (mg/L) using gas chromatography.

2.3.4. Effect of clarification time on light transmittance and methanol content

Take 100 mL of fresh honeydew juice each and place them in 6 conical flasks (250 mL). Adjust the pH to 5.5 using 20% citric acid, then add chitosan at a concentration of 0.04 g per 100 mL of juice. Clarify the mixtures in a 35°C water bath. At 10, 30, 50, 90, 120, and 180 minutes respectively, sample

and centrifuge to obtain the clarified liquid, then determine its light transmittance (T%). Add 0.2 mL of pectinase and 2 mL of cellulase to the remaining supernatant, perform enzymatic hydrolysis in a 35°C water bath for 2 hours, followed by distillation. Determine the methanol content (mg/L) using gas chromatography.

2.4. Orthogonal experiment

Based on the single-factor experiment results, an orthogonal experiment was conducted to screen the factors affecting chitosan clarification efficiency and investigate their comprehensive effects on clarification performance, thereby determining the optimal parameters for the clarification process. With methanol content as the primary index and light transmittance as the secondary index, a 4-factor, 3-level orthogonal experiment was designed, with the factors and levels as follows: chitosan addition amount(A) (0.02, 0.04, and 0.06 g/100 mL), system pH(B) (6.45, 5.50, and 4.50), clarification time (C)(10, 30, and 50 minutes), and clarification temperature (D)(30, 35, and 40°C). The experiment was then carried out.

3. results and discussion

3.1. Comparison between clarification effects of chitosan and pectinase

Direct clarification of honeydew juice using pectinase generates a relatively large amount of methanol, resulting in a high methanol content in the system. When chitosan is used for clarification, the flocculation of chitosan reduces macromolecules such as pectin in the honeydew juice. Direct determination of methanol in the clarified liquid shows a lower methanol content. The clarification effect of chitosan can be reflected by comparing the methanol data of honeydew juice after pectinase clarification with that of the system after chitosan clarification followed by pectinase hydrolysis.

As shown in Fig. 1, chitosan clarification can significantly reduce the methanol content in the system, with the methanol content decreasing by 53% compared to that before treatment. Therefore, studying the variation law of methanol in the chitosan clarification system has important application value.

3.2. Single-Factor experiments

3.2.1. Effect of aqueous chitosan addition amount on light transmittance and methanol content

As shown in Fig. 2, the light transmittance (T) of honeydew juice increases rapidly with the increase in chitosan addition amount. When the chitosan addition amount reaches 0.04 g/100 mL, the light transmittance (T) of honeydew

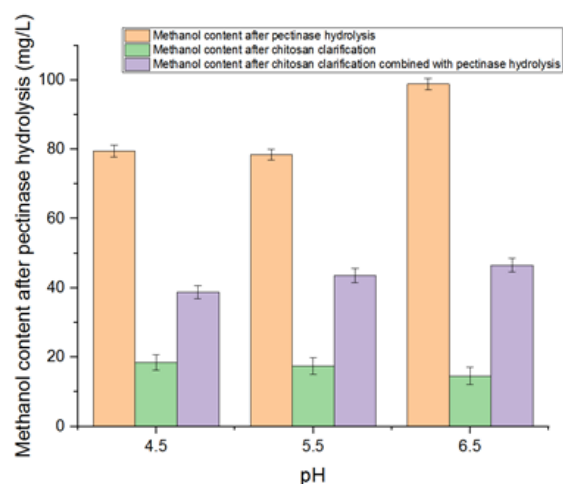


Fig. 1. Methanol content before and after clarification of honeydew juice with chitosan

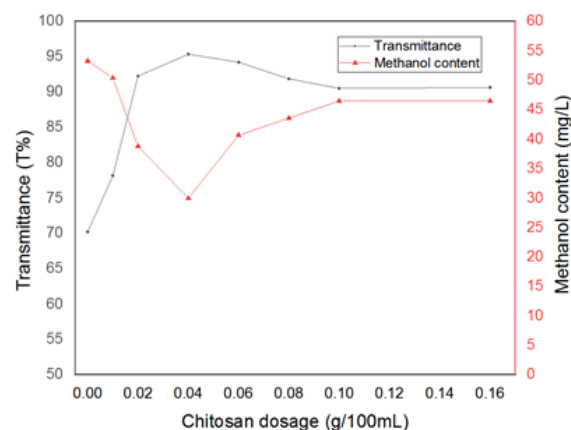


Fig. 2. Effects of chitosan dosage on light transmittance and methanol content of honeydew juice

juice reaches the maximum value of 94.17%. When the chitosan addition exceeds 0.04 g, the increase in solution light transmittance becomes insignificant with further addition of chitosan. This indicates that when the chitosan addition amount reaches 0.04 g/100 mL, the pectin sedimentation in honeydew juice is completed; adding more chitosan instead leads to a decrease in the light transmittance of honeydew juice.

The methanol content of the clarified honeydew juice after pectinase hydrolysis ranges from 30 to 60mg/L, and reaches the minimum value when the chitosan addition amount is 0.04 g/100 mL. This is because the clarification effect of water-soluble chitosan reduces the pectin content in honeydew juice, thereby decreasing the total amount of pectin available for decomposition by pectinase in the

later stage. It can be seen from Fig. 2 that when the chitosan dosage is 0.04 g/100 mL (where the light transmittance is maximum), the pectin content is the lowest and the methanol content is also at the minimum value, showing a corresponding relationship between the two.

3.2.2. Effect of aqueous chitosan clarification temperature on light transmittance and methanol content

As shown in Fig. 3, the light transmittance of honeydew juice shows a slow upward trend with the increase in clarification temperature, reaching the maximum value of 95.8% at 35°C, and then gradually decreases as the temperature continues to rise. This indicates that the pectin flocculation in honeydew juice is basically completed at 35°C. When the temperature is further increased, the activity of chitosan decreases, which affects the pectin sedimentation and thus leads to a decline in light transmittance.

The amount of methanol produced in honeydew juice changes little with the increase in temperature. When the temperature is between 35°C and 45°C, the methanol content remains at a relatively low level, only 30 – 31mg/L, which can be used as a reference for the selection range of process temperature. When the temperature ranges from 45°C to 50°C, the reason for the increase in methanol content may be that at higher temperatures, the residual pectin in honeydew melon decomposes more completely, resulting in a higher methanol production. When the temperature is relatively high, the methanol content is positively correlated with the decomposition degree of pectin.

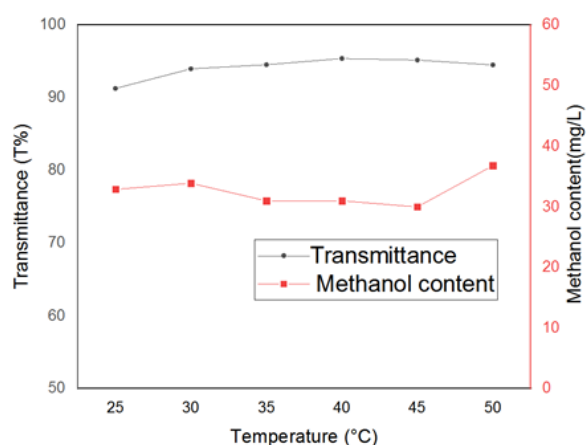


Fig. 3. Effects of clarification temperature on light transmittance and methanol content of honeydew Juice

3.2.3. Effect of initial pH value on light transmittance and methanol content

As shown in Fig. 4, the light transmittance of honeydew juice is relatively high when the pH value ranges from 4.0

to 5.5. Under the condition of increased acidity, pectin sedimentation is complete, thus resulting in high light transmittance. When the pH value is greater than 5.5, the light transmittance of honeydew juice decreases slightly as the pH value increases. This is mainly because the pH value of honeydew juice has a certain impact on the precipitation capacity of chitosan.

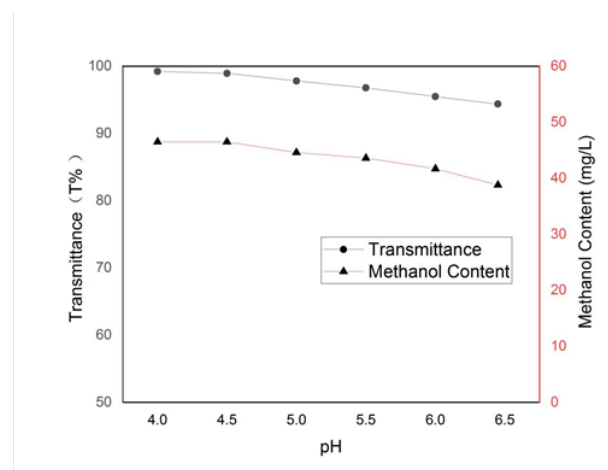


Fig. 4. Effect of Initial pH on Light Transmission and Methanol Content of honeydew Juice

The methanol content of honeydew juice after enzymatic hydrolysis shows a downward trend with the increase in the system's pH value. This indicates that pectinase is affected by acidity, and its ability to decompose pectin is weakened, leading to a gradual decrease in the methanol content of honeydew juice.

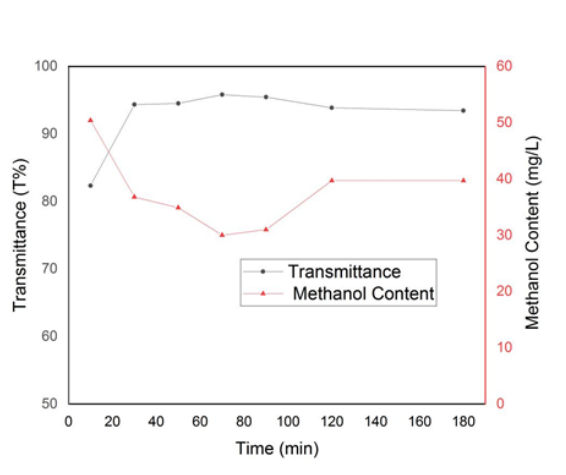
3.2.4. Effect of clarification time on light transmittance and methanol content

As shown in Fig. 5, the light transmittance of honeydew juice increases with the extension of time, reaching a maximum of 94.34% after 30 minutes of chitosan treatment, and then remains basically unchanged as time goes on. This indicates that the clarification effect of chitosan proceeds relatively rapidly, and the maximum clarification effect can be achieved in a short time. Further extending the clarification time cannot significantly improve the clarity of honeydew juice.

The methanol production in honeydew juice is affected by the residual pectin content in the chitosan clarification system and is negatively correlated with the light transmittance of the clarified solution. It can be seen from Fig. 5 that as the clarification time increases, the light transmittance rises while the methanol content shows a decreasing trend.

Table 1. Result of factors and levels of L9 (34) orthogonal test

Experiment No.	Chitosan Addition Amount (g/100 mL)	Initial Clarification pH (25°C)	Time (min)	Temperature (°C)	Methanol (mg/L)
1	0.02	6.45	10	30	52.2
2	0.02	5.5	30	35	38.8
3	0.02	4.5	50	40	36.3
4	0.04	6.45	30	40	30.6
5	0.04	5.5	50	30	33.2
6	0.04	4.5	10	35	48.2
7	0.06	6.45	50	35	38.6
8	0.06	5.5	10	40	54.8
9	0.06	4.5	30	30	47.5
K ₁	127.3	121.4	155.2	132.9	
K ₂	112	126.8	116.9	125.6	
K ₃	140.9	132	108.1	121.7	
k ₁	42.4	40.5	51.7	44.3	
k ₂	37.3	42.3	39.0	41.9	
k ₃	47.0	44.0	36.0	40.6	
R	28.9	10.6	47.1	11.2	
r	9.6	3.5	15.7	3.7	

**Fig. 5.** Effects of clarification time on light transmission and methanol content of honeydew juice

3.3. Results of orthogonal experiments

As shown in Table 1, based on the single-factor experiments on the clarification effect of chitosan treatment on honeydew juice and the amount of methanol produced, a comprehensive analysis of the interaction between various factors was conducted. A 4-factor, 3-level orthogonal experiment was designed with the factors including chitosan addition amount(A), initial pH value of the juice(B), clarification time(C), and clarification temperature(D). The goal of chitosan clarification is to reduce the pectin content, and the decrease in pectin content leads to a reduction in methanol content. In the orthogonal experiment, the lower

the methanol content, the better the clarification effect.

The results indicated that after chitosan clarification of the juice, the order of factors influencing the methanol content produced by pectin decomposition via pectinase was $B > D > A > C$, i.e., initial pH value > temperature > chitosan addition amount > clarification time.

The optimal process parameters for chitosan clarification were A2B1C3D3. Under these process conditions, experimental verification showed that the methanol content in honeydew juice was 28.6mg/L, which was lower than the methanol content obtained under other process schemes.

3.4. Comparison of partial components of honeydew juice before and after enzymatic hydrolysis and clarification

As shown in Table 2, compared with the original honeydew juice, the clarified juice after enzymatic hydrolysis had basically unchanged total sugar and vitamin C (VC) contents, increased acidity, a slight decrease in pH value, nearly complete removal of pectin, and an increase in methanol content from 13.2mg/L to 93.65mg/L.

When the honeydew juice was first clarified with chitosan and then subjected to pectinase hydrolysis, compared with the clarified juice obtained by direct pectinase clarification, its total sugar, total acid, pH, and VC contents remained basically unchanged, pectin was nearly completely removed, and the methanol content was 29.2mg/L. This methanol content was significantly lower than that in the system where enzymatic hydrolysis was performed with

Table 2. Changes of components of honeydew juice before and after enzymatic clarification

Sample	Total Sugar g/100g	Total Acidity g/100g	pH 25°C	VC mg/100g	Total Pectin	Methanol mg/L
Original Juice	9.3	0.135	6.5	18.6	0.34	13.2
Pectinase-Clarified Juice	9.1	0.353	4.1	17.3	0.01	93.65
Chitosan + Pectinase Clarified Juice	8.9	0.457	4.3	15.2	0.01	29.2

pectinase directly.

3.5. Discussion

The clarification effect of chitosan on honeydew juice is affected by chitosan addition amount, pH value, temperature, and clarification time. Chitosan is used to flocculate and precipitate proteins and pectin in honeydew juice; the mixture is then centrifuged to obtain clarified honeydew juice. After subsequent enzymatic hydrolysis with pectinase, the methanol content in the honeydew juice is determined.

3.5.1. Flocculation and clarification by chitosan

In juice processing, clarification is typically achieved through hydrolysis with pectinase and amylase, combined with treatment processes using bentonite, silica gel, and other materials. However, this type of process has complex operations, a long clarification cycle, and high material costs. More importantly, it cannot completely solve the problems of non-biological turbidity and browning that occur during juice processing.

Chitosan is derived from chitin via deacetylation. The amino groups on its molecule can be protonated to carry positive charges, making it currently the only cationic polymer in food [14]. When added to fruit pulp, chitosan can interact with negatively charged proteins, cellulose, and pectin in the pulp to flocculate them. Specifically, chitosan is first adsorbed onto particles in the juice through electrostatic interactions, and at the same time, it can form bridges between different particles. Subsequently, it forms flocs with pectin, thereby achieving the clarification of the juice [15]. Therefore, chitosan is an important chemical clarifying agent and is commonly used for the clarification of juices, beer, beverages, and other products [16].

3.5.2. Control of methanol content

Methanol is a colorless, transparent liquid with a slight alcoholic odor, high solubility in water, and extremely high toxicity. It is a harmful component in edible alcohol and one of the most important hygiene indicators. Methanol significantly anesthetizes the nervous system and has toxic side effects on the optic nerve and retina; the metabolites of methanol—formaldehyde and formic acid are 30 times and

6 times more toxic than methanol, respectively. Formaldehyde and formic acid can cause disorders of blood circulation and impairments to the coenzyme system in the body, leading to hypoxia in cerebral cortex cells and subsequent occurrence of a series of neurological symptoms [17, 18].

Therefore, China has strict upper limits on methanol content in Baijiu (Chinese liquor). For distilled spirits and blended spirits brewed with grain cereals as raw materials, the methanol content should be < 600mg/L (calculated based on 100% alcohol by volume); for those brewed with other raw materials, the methanol content should be < 2000mg/L (calculated based on 100% alcohol by volume) (Ministry of Health of the People's Republic of China, 2012).

3.5.3. Effect of chitosan clarification on methanol content in honeydew juice system

Water-soluble chitosan flocculates with pectin, and centrifugal separation is performed to achieve juice clarification. By adjusting process conditions, the degree of pectin removal from honeydew juice is initially determined through changes in light transmittance. For soluble pectin, pectinase is used to promote its complete decomposition, and the methanol produced during the pectin decomposition process is determined. After chitosan clarification, the lower the residual pectin content, the less methanol is produced in the subsequent stage. Additionally, the lower the pectin content, the higher the light transmittance of the clarified honeydew juice. Therefore, methanol content is positively correlated with soluble pectin content and negatively correlated with the light transmittance of the clarified juice. For detailed discussion, refer to the analysis in Figs. 2 to 5. The main factor contributing to the significant reduction of methanol content in the honeydew juice system via chitosan clarification is the decrease in pectin content in the juice, which is confirmed by experimental data.

4. conclusions

With the methanol content of honeydew juice as the primary research target, through single-factor experiment analysis and orthogonal experiment verification, the optimal process conditions for chitosan clarification of honeydew juice were determined as follows: chitosan addition

amount of 0.04 g/100 mL, pH = 6.45 clarification temperature of 35°C, and clarification time of 30 minutes. After subsequent centrifugation at 4800r/min for 10 minutes, the methanol content was 28.6mg/L, and the light transmittance could reach 96.35%. Pectin and proteins, which affect the long-term storage stability of honeydew juice, were largely removed during the clarification process, significantly improving the stability of the juice while maintaining a low methanol content.

Thus, the chitosan clarification method not only clarifies honeydew juice and well retains the main active components in the juice but also achieves the goal of reducing methanol content in honeydew juice.

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