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Effect of Corn Starch's Transformation Rate on the Quality of Papaya Wine

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Abstract. The purpose of this experiment was to study the effect of corn starch's transformation rate on the quality of papaya juice and discuss the its role in the fermentation process of papaya juice. Papaya grown in Lincang was taken as experimental material. During the period of 2017-2018, five repetitive experiments on gelatinization, liquefaction and saccharification of maize starch were carried out, respectively. Pure saccharified liquid, fermentable sugar used by yeast, was extracted by coarse filtration of gauze and filtration of suction filter. In 2017, the activity conditions of alpha-amylase and glucoamylase were determined. Four different saccharification methods including double enzymatic method, acid hydrolysis method, enzymatic hydrolysis method and acid enzymatic method were selected for experiments, respectively. The optimum method on saccharification of corn starch was determined according to the sugar content and yield of saccharified solution. The saccharified liquid obtained was added to peeled and non-peeled papaya juices for fermentation, respectively. During the experiment, the quality indexes, such as total sugar, total acid, tannin, anthocyanin and chroma were determined, respectively. In 2018, the transformation experiments of corn starch in corn flour, fresh corn granules and mature corn granules were meanwhile carried out. The best raw material for saccharification was selected through the total sugar content and the extraction rate of saccharification solution. The optimum conditions of enzyme activity were determined as pH 6, temperature 70 °C in α-amylase, pH 5 and temperature 60 °C in saccharifying enzyme. The yield of saccharified solution of mature corn granules was 60%, the sugar content was 193.64 g/L. The double enzymatic saccharification obtained 46 g/L sugar content and 46.60% yield of saccharified solution. Therefore, mature maize granules with double enzymes method were the best choice for saccharification experiments, which increased not only the sugar content and the yield of saccharified solution, but also made the operation simple and filter easy. The saccharified liquid extracted by mature corn granules combining with double enzymatic method could promote the fermentation of papaya wine, which, on the one hand, could protect the color of papaya wine, on the other hand, improve the flavor of papaya wine, and make papaya wine with unique honey and caramel flavor.

INTRODUCTION

Corn is the third largest food crop in the world after wheat and rice. The output of maize in China is next to that of America, and the annual sown area and total yield account for more than 15% in the world. It is an innovative technology that corn saccharified liquid is added to papaya juice for fermentation, which can be applied in practice in the future. The common method, starch sugar obtained from corn, is to extract the starch in corn before processing it into starch sugar.

At present, both wet and dry methods are widely used in the separation and purification of corn components, which have been industrialized in large-scale production at home and abroad. Wet process refers to the pretreatment method of maize raw materials in starch industry. It is to separate germ, fiber and protein from soaked corn in wet water after

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grinding roughly and finely, respectively, so as to obtain high purity of starch products. While dry method can separate the germ and fiber, meanwhile get low fat of corn flour by grinding, screening, air separation method without soaking in a lot of warm water.

The rise of wet processing is mainly due to the purity of starch, which can meet the processing needs of medicines and special fermented products with high recovery of corn protein, oil and gluten by-products, and considerable overall economic benefits. Its investment is higher than two times that of dry process. However, the weakness of dry processing is also quite prominent. For example, the recovery of corn oil of wet method is more than twice that of dry method when starch sugar is prepared, however the protein in corn starch is basically not separated. Fruit wine is usually fermented with fruit juice and sugar, which have single taste and a light taste [1].

Four saccharification methods have their advantages and disadvantages. The advantages of acid hydrolysis are simple process, short hydrolysis time and large production capacity of equipment. The disadvantages are high temperature and high pressure, acid corrosion, more side reactions and uniform size of starch granules. The advantages of double enzymes are mild reaction conditions, low requirements for starch liquor, less side reactions and purified sugar liquor. The disadvantages are longer production cycle and more complex equipment. The advantages of enzymatic method are simple process, short hydrolysis time, high production efficiency and quick turnover of equipment; the disadvantages are that there are many by-products, which affect the purity of sugar solution, and the general DE value is only about 90%. The starch raw materials are strictly required, crude starch cannot be used, only refined starch with high purity can be used; the advantages of enzymatic acid method are short hydrolysis time, high production efficiency and fast turnover of equipment. The length and by-products affect the purity of sugar solution. Combining their advantages and disadvantages, the actual operation of saccharification was carried out, and the final yield of saccharified solution was calculated, and the best scheme was selected according to the yield of saccharified solution for experiment.

The double enzymatic saccharification of maize meal is the deep hydrolysis of starch by amylase and glucoamylase to obtain the hydrolysate of glucose. The sugar solution is also rich in amino acids such as lysine and serine, B vitamins and a small amount of oligosaccharides with rich nutrients. Compared with the traditional technology, the papaya wine produced by adding saccharified liquid extracted from corn flour instead of sucrose into papaya juice for fermentation has not only fresh taste and mellow, but also high nutritional value and good application effect [2].

Generally, the research on maize starch only focuses on the activity of amylase and the extraction of polysaccharides from starch. However, there are still some problems in the experiment extracting saccharified liquid from maize starch, such as slow speed and long time-consuming. In this experiment, corn flour and fresh corn granules in Chuxiong market, as well as mature corn granules in Shandong were took as the main test materials. The effects of alpha-amylase and glucoamylase on the main quality traits of maize starch are studied through transformation treatment of corn starch and disparate raw materials of saccharification under different conditions to explore the main role of saccharified liquid in the fermentation of papaya juice, which provide theoretical basis for the production of high quality papaya wine.

MATERIALS AND METHODS

Materials

Acid papaya, in Lincang, Yunnan. Corn flour, Chuxiong market, in Chuxiong, Yunnan. Fresh corn granules, Chuxiong market, in Chuxiong, Yunnan. Mature corn granules, in Jiaxiang county, Jining city, Shandong.

The experiment was conducted in laboratory of grape and wine technology in Chuxiong Normal University from 2017 to 2018.

Design of Experiments

Design 1: The determination of activity conditions on alpha-amylase and glucoamylase

The yield of saccharified solution of corn starch after saccharification under two enzymes was determined under six temperature gradients, 40, 50, 60, 70, 80 and 90 °C, respectively. The optimum temperature and maximum tolerance temperature for alpha-amylase and glucoamylase were also selected for formal experiments to extract saccharified liquor. Similarly, six pH gradients were also set at pH 3, 4, 5, 6, 7 and 8 to study the yield of saccharified

solution under the combined action of the two enzymes, and optimize the optimal pH values suiting to the two enzymes for formal experiments.

Design 2: The determination of maize raw materials

Three different regions, maturity and states of corn starch, corn flour in Chuxiong market, fresh corn granules in Chuxiong market and mature corn granules in Shandong market, were selected for preparation experiments, respectively. The best corn raw materials were ascertained for formal experiments by determining the sugar content and the yield of saccharified liquid for three raw materials, respectively [3].

Design 3: Selection of the best saccharification of method. The saccharification experiments of corn starch were carried out by four methods: double enzyme method, acidolysis method, enzymatic acid method and acidolysis method [4].

Double enzyme method: soak 250 g corn particles in 50 ml of warm water at 45 °C for 30 min, then filter with gauze to obtain slurry. 250 g of corn slurry was added to 100 mL of distilled water for gelatinization. After gelatinization, 1 g/kg α -amylase was added, and then placed in a water bath to extract for 1 hour. After that, the temperature rose to 90 °C, the enzyme was inactivated for 30 min, cooled to room temperature, concentrated and filtered. supernatant, adding 1 g/kg glucoamylase for saccharification, performing suction filtration again after saccharification to obtain polysaccharide solution, concentrating under reduced pressure, filtering to obtain pure saccharified liquid, and weighing to obtain extraction rate.

Acidolysis: soak 250 g corn granule in 50 ml of warm water at 45 °C for 30 min, and then filt with gauze to obtain slurry. 250 g of corn slurry was added to 100 mL of distilled water for gelatinization. After gelatinization, liquefying under heating and pressure, adding hydrochloric acid, adjusting pH 6 to saccharification of acidolysis for 1 h, then concentrating under reduced pressure, then concentrating under reduced pressure, filtering to obtain pure saccharified liquid, and weighing to obtain its extraction rate.

Acid enzyme method: soak 250 g corn particles in 50 ml of warm water at 45 °C for 30 min, then filter with gauze to obtain slurry. 250 g of corn slurry is added with 100 mL of distilled water for gelatinization, hydrochloric acid is added after gelatinization, the pH is adjusted to 6 for hydrolysis for 1 h, then the corn slurry is placed in a water bath kettle for leaching, concentrated and filtered, supernatant is taken, saccharifying enzyme of 1 g/kg is added for saccharification, suction filtration is performed again to obtain polysaccharide solution, then decompression concentration is performed, pure saccharified liquid is obtained through filtration, and the extraction rate is obtained through weighing.

Enzymatic acid method: soak 250 g corn particles in 50 ml of warm water at 45 °C for 30 min, then filter with gauze to obtain slurry. Weighing 250 g of corn slurry, adding 100 mL of distilled water for gelatinization, adding 1 g/kg of alpha-amylase after gelatinization, then placing in a water bath for leaching, raising the temperature to 90 °C, inactivating the enzyme for 30 min, cooling to room temperature, concentrating and filtering, taking supernatant, adding hydrochloric acid, adjusting the pH to 6 for saccharification for 1 h, then concentrating under reduced pressure, filtering to obtain pure saccharified liquid, and weighing to obtain the extraction rate.

Design 4: Papaya juice was divided into two sets, peeling and non-peeling. The amount of saccharified liquid added to papaya juice was synthetically calculated by saccharified liquid concentration, 7% (v/v) of target alcohol content and quality of papaya juice. Then, yeast was added to the papaya juice for fermentation. The papaya wine with and without peeling with saccharified liquid was compared by determination of indexes after 15 days of fermentation.

Design 5: After 7 days, the contents of sugar, acid, pH, tannin, anthocyanin and chroma in above papaya juice were determined, and the effect of saccharified liquid on papaya wine was analyzed. The cause of change of each index was analyzed from whether the effect of papaya juice itself or yeast on alcohol fermentation, or adding saccharified liquid to a certain extent.

Amylase Hydrolysis of Corn Polysaccharide

The method referred to Aiyer [5] with a slight revision.

Corn granules 250 g were soaked in 50 mL warm water with 45 °C for 30 minutes before filtered with filter cloth to obtain the slurry. After the certain quality of amylase weighed accurately was added distilled water according to the weight ratio and activated in a water bath pot at a certain temperature for 30 minutes [6]. 250 g of corn slurry weighed was added into 100 mL distilled water for gelatinization. After gelatinization is over, 1 g/1000g of alpha-amylase was added, meanwhile the acidity and alkalinity of the solution was also adjusted before soaking in a water

bath. After inactivation for 30 minutes under 90 °C, the enzyme was cooled to room temperature. After filter was concentrated, extracted supernatant added 1 g/1000g of saccharifying enzyme to saccharify, and filtered again. The polysaccharide solution was concentrated under vacuum and precipitated with absolute ethanol. The precipitated substance was washed repeatedly with 95% ethanol and distilled water. The crude polysaccharide sample was dried and weighed. Finally, the extraction rate was obtained [7].

The formulas for calculating liquefaction yield and saccharification yield were as follows:

$$\text{Liquefaction yield (\%)} = \frac{\text{Quality of corn starch}}{\text{Quality of liquefied corn pulp}} \times 100\%$$

$$\text{Saccharification yield (\%)} = \frac{\text{Quality of corn starch}}{\text{Quality of the saccharification solution}} \times 100\%$$

Determination of Total Sugar

The determination for total sugar was mainly based on GB/T 15038-2006 standard [8].

Determination of Total Acids

The determination for total acids was mainly based on the GB/T 15038-2006 standard [8].

Determination of Tannin Content

Potassium permanganate titration [9:

5.0 mL of sample filtrate was absorbed in a triangular bottle before mixed with 10 mL water and 5 mL 2.5 mol/L H_2SO_4. After 5 minutes of micro-heating (50 °C), it was determined by 0.1 mol/L $KMnO_4$ to maintain a light pink color for 30 seconds without fading. The standard solution volume V1 was recorded.

5 mL of sample filtrate was extracted in 100 mL beaker before adding 3 g activated carbon to heat and stir for 10 minutes. A small amount of hot distilled water was employed to wash the residue filtered. Then collected filtrate added 5 mL 2.5 mol/L sulfuric acid was titrated to the end point with 0.01 mol/L $KMnO_4$ standard solutions, and recorded the volume V_2 consumed.

Calculation was carried out according to the following formula:

$$X = \frac{C \times (V_1 - V_2) \times 0.0416 \times 100}{m}$$

Where: X - tannin content, g/L; C - Molar concentration of $KMnO_4$, mol/L; V_1 - mL number of $KMnO_4$ consumed by titration sample, mL; V_2 - mL number of $KMnO_4$ consumed by tannin after activated carbon adsorption, mL; 0.0416 - 1 mL 0.01mol/L $KMnO_4$ solution mg number equivalent to tannin, mg; M - Grams number of sample, g

Determination of Anthocyanin Content

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

$$\text{A} = [(A_{510} - A_{700})_{pH1.0} - (A_{510} - A_{700})_{pH4.5}]$$

Anthocyanin concentration:

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

Where: (A_{510}-A_{700}) pH1.0 means difference in absorbance between sample solution with pH1.0 buffer at 510 nm and 700 nm; (A_{510}-A_{700}) pH4.5 means difference in absorbance between sample solution with pH4.5 buffer at 510 nm and 700 nm; MW=449.2 means molecular weight of cornflower - 3 - glucoside, mg/mol; DF= multiple dilution of sample solution; ε=26900 means the molar extinction coefficient of cornflower - 3 - glucoside, mol^{-1}; L = diameter of light path of the cuvette, 1 cm.

Colorimetric Determination

Spectrophotometry [11]:

(1) The pH value of wine samples was measured by pH meter.

(2) Preparation of buffer solution

A liquid: 0.2 mol/L disodium hydrogen phosphate. 7.12 g disodium hydrogen phosphate weighed was metered volume to 200 mL.

B liquid: 0.2 mol/L citric acid. 4.2 g citric acid weighed was metered volume to 200 mL at constant volume.

The buffer could be prepared by mixing A and B liquids and determined directly the pH value with a pH meter.

(3) After took in 25 mL colorimetric tube with big belly straw, 2 mL wine sample was diluted to 25 mL with buffer of the same pH value as papaya juice, and then the absorbance value was measured at lambda 420, lambda 520 and lambda 620 of spectrophotometer with 1 cm colorimetric dish (with distilled water as blank).

(4) Calculations: The sample measured should be placed in the colorimetric tank for 15 minutes, and then observe bubbles in the colorimetric dish. Bubbles should be driven out before colorimetric start. The absorbance values of lambda 420, lambda 520 and lambda 620 measured on the spectrophotometer would be added together and multiplied by the dilution factor of 12.5, which was the chroma. Result should reserve 1 decimal. The difference between the absolute values determined by parallel experiments should not exceed 0.1.

$$\text{Chroma} = (A_{420} + A_{520} + A_{620}) \times \text{Diluted multiples}$$

Statistical Analysis

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

RESULTS AND DISSCUSS

Determination of Optimum Conditions for Enzyme Activity

Under six different temperature gradient conditions, activity of α-amylase and glucoamylase were mainly determined by the yield of final concentration saccharified liquid [12].

As shown in Table 1, the liquefaction rate of corn starch adding 1g/kg of α-amylase increased gradually with the hoist of temperature, and the yield of liquefied solution reached the highest value, 46.60%, at 70 °C. After that, the yield of liquefied solution decreased gradually with the increase of temperature, showing an inverted U - shaped curve. Therefore, the optimal temperature for α-amylase hydrolysis was 70 °C, while the tolerant maximum temperature was 90 °C, which was consistent with the research of Baks et al. [13]. Therefore, six gradients, between 40 °C and 90 °C, were selected for the experiments. The saccharified rate of corn starch adding 1g/kg of saccharifying enzyme increased gradually with the increase of temperature, and the yield of liquefied liquid reached the highest number , 48.30%, at 60 °C. After that, the yield of saccharified liquid decreased gradually with the increase of temperature with an inverted U - shaped curve. Therefore, the optimum functional temperature for saccharifying enzyme was 60 °C, and the tolerable maximum temperature was 90 °C, which was consistent with the research of Rebroš et al. [14]. Therefore, six gradients, the lowest temperature 40 °C and the highest temperature 90 °C, were selected for experiments.

The process of starch saccharification must guarantee water absorption and expansion to a certain degree generally first before gelatinization. Followly,α- amylase was added at the dosage of 1 g/kg for liquefaction before collation of corn steep liquor. Finally, saccharification is carried out by adding 1 g/kg of saccharifying enzyme, while the final product of saccharification was changed from oligosaccharide to maltose and final glucose. Therefore, α-amylase and glucoamylase played a key role in the whole saccharification process, and the study of their enzyme activities plays a key role in the preparation of saccharified liquid.

TABLE 1. Determination of the optimum temperature of enzyme activity

	40 (°C)	**50 (°C)**	**60 (°C)**	**70 (°C)**	**80 (°C)**	**90 (°C)**
Alpha amylase (Liquefied liquid yield, %)	37.30±0.32	39.20±0.1	42.00±0.040	46.60±0.40	41.90±0.20	41.20±0.20
Saccharifying enzyme (Saccharification liquid yield, %)	39.00±0.20	43.20±0.40	48.30±0.20	45.80±0.40	39.20±0.40	43.60±0.40

The activity determination of α - amylase and glucoamylase depended mainly on the yield of final saccharified liquid under 6 types of pH condition.

As shown in Table 2, the liquefaction rate of corn starch adding 1 g/kg of α - amylase increased gradually with the ascending pH value. The yield of liquefied solution reached the highest at pH6, 42.60%. Thereafter, the yield of liquefied solution decreased gradually with the increase of pH value, showing an inverted U - shaped curve. Therefore, the optimal pH6 for α - amylase hydrolysis was selected, which was consistent with the research of Burhan et al.[15]. The saccharified rate of corn starch adding 1 g/kg glucoamylase increased gradually with the up pH value, and the yield of liquefied solution reached the highest one at pH5, 46.90%. After that, the yield of liquefied solution decreased gradually with the anabatic pH value with an inverted U - shaped curve. Therefore, the optimal pH value for α-amylase hydrolysis was 5, which was consistent with the research of Sun et al. [16]. During the saccharification process, the pH value affected directly the saccharified effect, especially the key control of pH value when different methods, such as acidolysis method, were selected for saccharification [17].

According to the above Tables 1 and 2, the optimum activity conditions of α-amylase were pH 6, temperature 70 °C, The optimum pH value of glucoamylase was 5 and the temperature was 60 °C. Saccharification could be stopped when the saccharification end point was reached through estimate without white precipitate after anhydrous ethanol was dripped into saccharified liquid [18].

TABLE 2. Determination of the optimum pH value of enzyme activity

	3	4	5	6	7	8
Alpha amylase (Liquefied liquid yield, %)	25.00±0.20	26.20±0.10	33.80±0.20	42.60±0.10	38.60±0.10	36.90±0.40
Saccharifying enzyme (saccharified liquid yield, %)	32.80±0.40	36.20±0.20	46.90±0.30	41.90±0.30	39.80±0.10	41.80±0.30

Determination of Corn Raw Materials

Three kinds of corn starches with different states and maturities were selected for experiments, and the optimal raw materials of saccharified liquid were determined through the concentration and yield obtained saccharified liquid.

As shown in Table 3, the saccharified liquid yield of corn starch was 46.40%, the yield of fresh corn granules was 27.70%, and the value of mature corn granules was 60%, comparing the saccharified liquid yield of fresh corn granules, corn starch and mature corn granules, the saccharified liquid yield of mature corn granules was the highest. The sugar content of corn starch saccharified liquid was 41 g/L, that of fresh corn granule saccharified liquid was 7 g/L, and that of mature corn granule saccharified liquid was 193.64 g/L. Comparing the sugar content of fresh corn granule, corn starch and mature corn granule saccharified liquid, the sugar content of mature corn granule saccharified liquid was the highest. To sum up, select of mature corn grains for saccharification was optimal way. This raw material was not only simple to operate and convenient to filter, but also the efficiency of saccharified liquid was very high. Corn contained a large number of nutritional and health-care substances, such as glutathione with an anti-cancer factor, as well as riboflavin and vitamins with a great effect on the prevention of heart disease and cancer [19]. In addition, corn accumulated also a large amount of plant cellulose, which could accelerate the elimination of toxins in the body. The saccharification test of corn as raw material integrated not only the flavor of corn into papaya wine, but also played a role in health care to a certain extent [20]. Therefore, saccharification test of corn could promote the quality of papaya wine.

TABLE 3. Determination of corn raw materials

	Corn starch	Fresh corn granules	Mature corn grain
Yield of saccharified liquid (%)	46.40±0.10	27.70±0.30	60.00±0.10
Sugar content (g/L)	41.00±0.03	7.00±0.06	193.64±0.03

Determination of the Best Saccharification Method

Four different saccharification methods were selected in the experiments, and the best saccharification method of corn starch was determined through the concentration and the rate of the saccharified liquid obtained.

As shown in Table 4, starch was converted into sugar by double enzyme method, acid hydrolysis method, enzyme hydrolysis method and acid enzyme method, the sugar content of which was 46 g/L, 28 g/L, 39 g/L and 16 g/L,

respectively. Thus the double enzyme method obtained the highest sugar content. Additionally, conversional rate, the yield of saccharified liquid, of starch into sugar by four methods, was 46.60%, 45.80%, 46.10% and 39.70%, respectively, and the highest yield of saccharified liquid belonged to also double enzyme method. Comprehensive selection of double enzyme method for saccharified liquid extraction could not only improve the yield of saccharified liquid, but also materials were easy acquisition in nature.

Research showed that the saccharified liquid prepared by double enzyme method had less protein, starch, ash residue and high yield, and better water holding capacity than the products prepared by traditional acid-base method. At the same time, sodium chloride concentration, sucrose concentration and pH in conventional food system had little influence on the water-holding capacity of dietary fiber in corn bran, so that it could give full play to physiological activity in food processing [21]. The second was acidolysis [22], which could also effectively obtain sugar. The main processes were as follows: raw materials (starch, water and hydrochloric acid) → pulp mixing → saccharification by steam → cooling → neutralization (Na_2CO_3) →decolorization (activated carbon) → filtering in removing impurity → sugar solution. Advantages of this method were simpler process, shorter hydrolysis time, higher production efficiency and faster turnover of equipment. The disadvantage is that there are many by - products, which affect the purity of sugar solution. Generally, the dextrose equivalent was only about 90%, which required strict starch raw materials. Crude starch could not be used except for refined starch with higher purity. According to advantages and disadvantages combined with reality, as well as the sugar content and the yield of saccharified liquid in the experiment, the formal experiment was carried out by double enzyme method.

TABLE 4. Comparison of sugar content and yield of saccharified liquid to four saccharification methods

	Double enzyme method	Acid hydrolysis	Enzymatic acid method	Acid enzyme method
Sugar content (g/L)	46.00±0.10	28.00±0.10	39.00±0.10	16.00±0.20
Yield of saccharified liquid (%)	46.60±0.10	45.80±0.30	46.10±0.10	39.70±0.20

Effect of Saccharification Solution on Papaya Juice and Papaya Wine

Papaya juice was divided into two types according to with peel (WP) and without peel (WOP) to ferment. After 15 days of fermentation, the indexes of peeled and non - peeled papaya juice with saccharified liquid were measured and compared.

As shown in Table 5, after fermentation, the sugar contents, acid content, tannin content, chromaticity and anthocyanin content of papaya wine in WP and saccharified liquid were greater than that of the WOP and saccharified one, respectively. According to this analysis, the acid, tannin and anthocyanin in papaya came mainly from the pulp of papaya. Therefore, the pulp of papaya was darker in color than the peel. Therefore, the experiment with peeled raw materials could promote greatly the fermentation of papaya wine and made the papaya wine contain honey flavor and caramel flavor [23]. The addition of saccharified liquid had certain influence on the fermentation of papaya wine, which protected the color of papaya wine to a certain extent. On the other hand, the active microorganisms in the saccharified liquid were easy to cause diseases of papaya wine and terminate the fermentation. After tasting, the finished wine had no honey taste and other aroma, and the wine body was slightly light, which did not reach the expected alcohol content[24].

TABLE 5. Effect of Selecting Mature Corn Granules in Formal Experiments

	Sugar (g/L)	Acid (g/L)	pH	Tannin (mg/100g)	Anthocyanin (mg/g)	Chroma	Alcohol (%, vol)
Shelling + saccharified liquid	7.50	19.50	3.50	0.34	0.46	37.01	6.80
Keeping skin + saccharified liquid	7.00	17.62	3.57	0.03	0.25	16.80	6.90

After 7 days of fermentation with peeled papaya juice, the differences between saccharified liquid and sucrose as well as among various indexes from fermentation were compared, and the reasons for the differences were analyzed.

In Table 6, the sugar content, acid content, temperature and specific gravity in the papaya wine with saccharified liquid was less than that with sucrose, whereas the chromaticity and pH in the papaya wine with saccharified liquid was greater than that with sucrose, respectively. Therefore, the addition of saccharified liquid could promote the

fermentation very quickly, but the fermentation stopped quickly yet due to the low sugar content. In addition, a small amount of sediment was generated at the bottom of the fermentation tank due to the less pure extraction of saccharified liquid [25], which affected the clarification of papaya wine and promoted darker color, further protected the color of papaya wine and maintained the normal fermentation. Papaya wine has certain caramel flavor and certain acid to support its soul.

TABLE 6. Determinations of basic indexes of papaya wine

	pH	Temperature (°C)	Proportion	Sugar (g/L)	Acid (g/L)	Chroma
Sucrose	4.30	17.50	1.096	18.00	7.87	10.09
Saccharified liquid	4.47	17.00	1.048	14.00	6.38	18.51

Corn starch is also called cornstarch, the common name is six valley powders with a yellowish white powder, which is prepared through many steps such as crushing, sieving, settling, drying and grinding after the corn is soaked with 0.3% sulfurous acid. Ordinary products contain a small amount of fat and protein. Corn starch contains a large amount of sugar. The saccharified liquid extracted from corn starch can not only replace the original sucrose, but also improve the flavor of brewed papaya wine. At the same time, corn starch can also improve the color of papaya wine, which transforms seemingly immature green into mature golden yellow [26].

Chaenomeles speciosa, known also as Zou papaya, is the fruit of chaenomeles lagenaria, papaya plants, rose family. Chaenomeles sinensis grows mainly in a warm moist mountain region of the subtropics, and is cultivated in some provinces, such as Shandong, Jiangsu, Zhejiang, Anhui and Yunnan in China. The distribution area of Yunnan Province was rich in acid papaya [27]. Cultivation area of Chaenomeles Chaenomeles in Yunnan takes Dali Prefecture as the center and can extend southward to Jiangcheng, Pu'er, Shuangjiang and northward to Tibet and Sichuan. A variety of regional climate, located in the Yunnan-Kweichow Plateau, has created conditions for the diversification of fruit crops in Yunnan. Chaenomeles sinensis belongs to angiospermae, dicotyledoneae, rose family, papaya, which is mainly characterized by low sugar and high acid. In order to enable normal fermentation of papaya, some methods including biological deacidification and chemical deacidification need be adopted to reduce the acidity of papaya raw materials to achieve the acidity conditions of fermentation.Compared to other methods, the addition of saccharified liquid can achieve anticipant effect, promot the complete fermentation of papaya wine further.

At present, the common papaya wine was fermented by the traditional brewing method adding sucrose [28]. In this study, purified saccharified liquid was extracted from mature corn grains by double enzyme method and added into papaya juice for fermentation, which was in sharp contrast to previous research. The results of this experiment showed that the fermentation effect saccharified liquid was stronger than that of sucrose. Additionally, saccharified liquid could also produce caramel flavor and honey flavor in papaya wine during fermentation. At the same time, the addition of saccharified liquid had certain influence on the fermentation of papaya wine, which was mainly reflected in the protectoral chromaticity of papaya wine to a certain extent. However, due to the active microorganisms in saccharified liquid, papaya wine was prone to produce diseases and terminate the fermentation. Therefore, appropriate sulfur dioxide should be added to inhibit the activities of microorganisms and ensure the growth of yeast, so as to avail complete fermentation and the realization of target alcohol content.

CONCLUSIONS

Saccharified liquid from mature yellow corn grains in Shandong province, was added into the papaya juice to promote the fermentation to a certain extent. The reduction of acidity could accelerate the fermentation speed, and change the original flavor in the papaya juice. The experimental study showed that the optimum activity conditions were of pH6, temperature 70 °C on α - amylase, and pH5 and temperature 60 °C on glucoamylase. Corn starch was saccharified with double enzymes. The sugar content was 46 g/L and the yield of saccharified solution was 46.60%. Corn starch, fresh corn granules and mature corn granules were used as raw materials. Mature corn granules were finally selected as the best raw material for starch conversion sugar. The yield of saccharified solution was 60%, and the sugar content was 193.64 g/L.

In this experiment, two kinds of papaya juice, peeled and non - peeled, were selected for fermentation. By measuring the total sugar, total acid, tannin, anthocyanin, color, pH and alcohol content, results were obvious that the acid came mainly from the pulp of papaya, tannin and anthocyanin existed mainly in the pulp. Thus, the pulp color of papaya was darker than that of the peel, so the experiment with peeled raw materials and selecting saccharified liquid could promote greatly the fermentation of papaya wine. However, low sugar content stopped fermentation quickly,

and due to less pure saccharified liquid extraction, a small amount of sediment is generated at the bottom of the fermentation tank, which affects the clarification of papaya wine and makes its color darker, thus protecting the color of papaya wine and making the fermentation normal. Papaya wine taking saccharified liquid as mature yellow corn grains could generate certain caramel flavor and certain acid to support its framework.

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