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Low-cost recycling production of pectinase to increase the yield and quality of Muzao jujube juice by *Aspergillus niger*

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Abstract: A low-cost recycling system for the production of pectinase was built to improve the yield and quality of Muzao date juice and to reduce production costs. An *Aspergillus niger* strain, Gyx086, was selected from 20 *A. niger* strains on potato agar plates containing Muzao date residues, and the strain was used to produce an enzyme cocktail with high pectinase activity using Muzao date residues as the medium. The high pectinase activity level was therefore used in the production of Muzao date juice. Under optimized enzymatic hydrolysis conditions, a date juice yield of 92.88% was obtained, which was 11% higher than the control, and was not significantly different from a commercial preparation. Further storage trials indicated that the clarity, viscosity, storage stability, and soluble solid content could also be greatly improved by enzymatic treatment. These results suggested that the cheap experimental preparation could effectively

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replace the commercial preparation used for the production of date juice. This is the first systematic report detailing the production of Muzao date juice using a cheap and effective pectinase cocktail, which could promote the development of the date juice industry. © 2019 Society of Chemical Industry and John Wiley & Sons, Ltd

Supporting information may be found in the online version of this article.

Keywords: Muzao date; juice yield; *Aspergillus niger*; enzymatic hydrolysis; pectinase

Introduction

Ziziphus jujuba Mill. (common jujube) is one of the most important *Zizyphus* species in the buckthorn family, Rhamnaceae.[1] More than 700 varieties and cultivars of the fruit are distributed in different regions of China,[2,3] which is the largest producer of the common jujube (Chinese date), at about 8 522 000 tons in 2017.

Jujube fruit is widely consumed as food and in traditional Chinese medicine.[4,5] Usually, the fresh dates are only used for food and the dried dates are used for food and medicine because of their better storage ability. Jujube fruit was used as food due to its high nutritional value with a high sugar, protein, free amino acid, vitamin, and mineral content,[2,6–8] and as functional food or Chinese medicine due to its multiple active components, such as triterpenic acids,[9] flavonoids,[10,11] phenolics,[10,11] nucleosides, and nucleobases.[12] Thus, dates were usually considered to treat myriads of ailments including anorexia, fatigue, and loose stools in deficiency syndromes of the spleen and of hysteria in women.[12]

Jujube was defined as an underutilized crop by the International Centre for Underutilized Crops (ICUC).[13] Recent research on jujube fruit has mainly considered the analysis of nutritional and active components;[2,3,6–12] a few articles have considered its flavor.[8,14] However, the downstream processing of jujube is not well documented. The objective of the current work is to produce effectively date juice that may be used extensively as an additive for beverages, dairy products, or other products.

To facilitate the release of nutrients and / or active substances, most of which are the intracellular materials from plant cells, it is usually necessary and effective to break the cell walls to enhance the yield.[15] The conventional method of extracting date juice in China is to extract the dried jujube powder using water with a solid / liquid ratio at 1:10, 60 °C for 2 h. Then the mixture is divided into two fractions, the date juice and the residues. However, mechanical crushing consumes a large amount of electricity and has a very limited effect in reducing the date size due to the large viscosity of the date powder, resulting from its high sugar content. Thus, the juice yield of dates is very low and the product cost is high. An enzyme cocktail can hydrolyze components of the plant cell wall to facilitate the release of intracellular material with the catalytic conversion of polysaccharides into oligosaccharides and monosaccharides.[16] Moreover, enzyme pretreatment is conducted in mild conditions, which are more eco-friendly than physical and chemical methods.[16,17] To degrade plant cell walls, the enzyme cocktail generally includes cellulase, hemicellulase, pectinase, etc.,[18] because the plant cell wall is a complex polymer composed of cellulose, hemicellulose, lignin, and other components.[19] Nevertheless, commercial enzymes are not only expensive but also uncertain in effectiveness due to their enzyme systems mismatching the specific material used. We therefore aimed to select safe and effective strains to obtain an economically feasible and effective enzyme cocktail, and then to further conduct effective enzyme hydrolysis of dates to improve the yield and quality of date juice. Valueless date residues, which amount to about 550 000 tons in China each year, were used as the medium fermented by *A. niger* for the production of the economically feasible and effective enzyme cocktail. The newly developed technology of recycling-production of pectinase is a low-cost and beneficial method for increasing the yield and quality of Muzao jujube juice.

Materials and methods

Materials

Muzao dates were harvested in October 2017 from Linxian, Shanxi province, China. Fresh dates were dried using an air-drying oven at 70 °C and 25–28% of the water content remained due to the high sugar content. The cooled dates were stored in airtight plastic bags until they were used. Date residues with size 60 meshes were supplied by the Shanxi Zhongying Redzao Co., Ltd., China

Twenty strains of *Aspergillus niger* (*A. niger*) were used in this research. The Gyx086 and Gyx017 strains were isolated from ancient ginkgo tree soil in Anlu city, China (Wang

et al.[15]). The other strains, isolated from forestry soil in Thunder Bay, Canada, were stored in the lab. *Penicillium ramulosum* N1 was used as the control.

Evaluation of hydrolysis ability of *A. niger* strains

The hydrolysis ability of 20 *A. niger* strains was evaluated by a Congo red staining method described by Wang, *et al.*,[16] with slight modifications. In brief, 1 µL of spore suspension (1×10^8 per milliliter) was inoculated in the center of a 9.0 cm plate with 15 mL potato agar containing 0.05% (m/v) of date residues. These plates were incubated at 30 °C for 48 h. After the incubation, the plates were flooded with 10 mL of 0.1% Congo red solution and a dye–polysaccharide interaction was executed for 10 min. After pouring out the dye, the plate was washed three times with 10 mL distilled water and the diameters of the fungal colony (d) and halo region (D) were measured using a millimeter scale. The hydrolysis activity was calculated as $(D/d)^2$.

Medium preparation and single factor experiment

All the experiments were conducted in 250 mL Erlenmeyer flask with 50 mL of broth containing 5% of solid date residues and 0.07% of $(NH_4)_2SO_4$. This medium was autoclaved at 121 °C for 20 min, cooled to room temperature, and 1.0 mL of spore suspension was mixed into the medium under NU-340 laminar airflow workstations (NuAire, USA). Three levels of inoculum size were set as adding 1 mL of spore suspension with 10^5 10^7 and 10^9 spores/ml, four levels of temperature condition set as 27 °C, 30 °C, 33 °C, and 36 °C and pH levels were set as 3, 5, 7 and 9. During fermentation, enzyme cocktail samples were taken every 2 days for enzymatic activity analysis.

Enzyme assay

The enzymatic activities of polygalacturonase (PG), and xylanase, and filter paper activity (FPase) were measured by a high throughput analysis method described by Wang, *et al.*,[16] in 96-well plates. The chemical standards, polygalacturonic acid (Sigma, USA), beechwood xylan (Megazyme, Ireland) and Whatman No. 1 filter paper (Whatman, England) were used as the substrate for enzymatic activity analysis of PG, xylanase, and FPase, respectively. In brief, 10 µL of the moderately diluted crude enzyme was mixed with 20 µL of substance solution (1%, pH 5.0) in each well of the microplate. The reactions were executed in the water bath at 50 °C for 10 min (FPase for 20 min), cooled and 60 µL of the 3,5-dinitrosalicylic acid (DNS) reagent was added, and this was heated in boiling water for 5 min. The absorbance at 540 nm was recorded to calculate the released amount of reducing sugar. Enzyme activities were expressed in international units (IU), as the amount of enzyme that releases 1 µmol of glucose, xylose, or galacturonic acid in 1 min.

Enzymatic hydrolysis of Muzao date and juice yield calculation

The dried Muzao dates were shattered through a mesh size 20 screen before being used for juice production. Enzymatic hydrolysis was conducted in 250 mL Erlenmeyer flasks with 10 g of Muzao date fruit pulp in each flask. These flasks were placed in VWR® Incubating Orbital Shaker, Model 3500I (Henry Troemner. LLC, USA) with a rotation speed of 150 rpm. After the enzymatic hydrolysis, the mixtures were separated by a centrifugal operation at 13800 g for 10 min. The supernatant was carefully poured out and weighed. The juice yields were calculated by the following equation:

$$\text{juice yield}\,(\%) = \left[\frac{\text{supernatant juice weight}\,/}{(\text{date pulp weight} + \text{added liquid weight})}\right] \times 100$$

Assay of clarity, viscosity, flavon content and browning degree

Date juice clarity was expressed as the luminousness at 650 nm by a BioTek Epoch microplate spectrophotometer (BioTek, USA). Viscosity was measured by a KV5000 Ubbelohde viscometer (Nasrem, China) and calculated by the following equation:

$$\text{viscosity}\,(cp) = \text{time}\,(\text{min}) \times 60 \times 0.01\,(\text{centistokes}\,/\,\text{s})$$

Flavone content was determined using the spectrophotometric method described by Jia *et al* [20] and the method was improved to operate in a microwell plate. Briefly, a 20 µL sample was mixed with 80 µL deionized water and then 10 µL 5% (w/v) $NaNO_2$ was added. After 6 min of reaction, 10 µL of 10% aqueous aluminum chloride solution was added and then after 6 min, 90 µL of 1 M sodium hydroxide was added. After 13 min, the absorbance was determined at 510 nm. The flavone content was counted by the standard curve using chemical standard rutin (Yuanye, China). The degree of browning was expressed as absorbance at 420 nm[21] because the browning product has the largest absorbance at this wavelength.

Box–Behnken design (BBD) and statistical analysis

The production of pectinase and juice yield was optimized using a response surface methodology (RSM) – refer to our previous description.[22] The BBD with three factors and three levels was executed based on the results of the single factor experiment shown in Table 2 and Table 4. SYSTAT 12 (Systat Software, Inc., USA) was used to generate the BBD matrix and analyze the experimental data, describing the response surface, and drawing the contour maps. The goodness of fit of the second-order polynomial model equation, the determination coefficient R^2, and the lack of fit were indicated by an F test at the 5% level of significance. The most optimized conditions were also decided by the SYSTAT 12 software optimizing program. The statistical analysis of data was carried out using one-way ANOVA with the SAS system for Windows 8.02 (SAS Institute Inc., USA). Duncan's multiple-range test was selected as the comparative method at the 0.05 significance level.

Results and discussion

Screening high-effective *A. niger* strain using date residues

Aspergillus niger is one of the most important species in the food industry. It is widely used for the preparation of various enzymes, such as cellulase, hemicellulase, and pectinase.[15,16,23,24] *Aspergillus niger* is considered to be a safe food strain.[25] To select an *A. niger* strain with high hydrolysis ability, a total of 20 *A. niger* isolates were investigated by observing clear zones using Congo red staining on agar plates containing 0.05% date residues (Table 1). All of the *A. niger* strains formed big colonies with diameters from 18.3 mm to 40.3 mm in 48 h of growth, which indicated that these fungal isolates were able to grow faster on agar plates with date residues compared to the control. There were halos of 28.3–46.3 cm around the fungal colonies, which suggested that all the *A. niger* strains could effectively degrade polymers in date residues to small molecules that could not be stained by Congo red compared to the control. Nevertheless, *A. niger*

Table 1. Hydrolysis ability of 20 *Aspergillus niger* strains on the potato agar plates including 0.1% of date residues.

No.	Code	Halo diameter (D, cm)	Colony diameter (d, cm)	Hydrolysis ability $(D/d)^2$
1	*Penicillium*N1	0.80 ± 0.00^{a}	0.76 ± 0.05^{a}	1.11 ± 0.16^{a}
2	TBA18-3	3.90 ± 0.88^{b}	3.38 ± 0.97^{efgh}	1.43 ± 0.36^{ab}
3	TBA18-A7	3.78 ± 0.23^{b}	2.98 ± 0.13^{d}	1.61 ± 0.12^{bc}
4	Gyx017	5.18 ± 0.36^{i}	4.03 ± 0.41^{k}	1.67 ± 0.23^{bc}
5	TBA18-5-1	4.98 ± 0.19^{ghi}	3.68 ± 0.24^{hij}	1.84 ± 0.18^{bcd}
6	TBA18-A5	4.78 ± 0.25^{fgh}	3.53 ± 0.26^{fghij}	1.86 ± 0.29^{bcd}
7	TBA18-9	5.10 ± 0.19^{i}	3.78 ± 0.43^{ijk}	1.86 ± 0.26^{bcd}
8	TBA18-8	5.03 ± 0.13^{ghi}	3.65 ± 0.14^{ghij}	1.90 ± 0.12^{bcd}
9	TBA18-12-1	4.75 ± 0.14^{efg}	3.43 ± 0.13^{efghij}	1.93 ± 0.10^{cd}
10	TBA18-1-1	5.25 ± 0.33^{i}	3.83 ± 0.43^{jk}	1.93 ± 0.41^{cd}
11	TBA18-8-1	5.08 ± 0.24^{hi}	3.63 ± 0.33^{ghij}	1.98 ± 0.22^{cd}
12	TBA18-1	4.65 ± 0.09^{ef}	3.30 ± 0.11^{defg}	1.99 ± 0.15^{cd}
13	TBA18-11	5.03 ± 0.13^{ghi}	3.53 ± 0.15^{fghi}	2.04 ± 0.12^{cd}
14	TBA18-A6	4.65 ± 0.18^{ef}	3.13 ± 0.10^{de}	2.22 ± 0.20^{d}
15	TBA18-4	5.18 ± 0.36^{i}	$3.20 \pm 0.21^{d\,ef}$	2.65 ± 0.47^{e}
16	TBA18-A1	4.05 ± 0.14^{bc}	2.10 ± 0.15^{c}	3.77 ± 0.59^{f}
17	TBA18-3-1	3.95 ± 0.30^{bc}	2.03 ± 0.27^{c}	3.88 ± 0.60^{f}
18	TBA18-A4	3.83 ± 0.13^{b}	1.88 ± 0.10^{bc}	4.19 ± 0.47^{fg}
19	TBA18-6	4.23 ± 0.13^{cd}	2.00 ± 0.00^{bc}	4.47 ± 0.27^{g}
20	TBA18-10	4.08 ± 0.10^{bc}	1.68 ± 0.10^{b}	5.95 ± 0.12^{h}
21	Gyx086	4.45 ± 0.21^{de}	1.83 ± 0.17^{bc}	6.10 ± 0.13^{h}

Notes: Values represent mean ± SDs (n–8). Different letters indicate significant differences ($P < 0.05$) according to the Duncan multiple comparative analysis.

Table 2. Box–Behnken design matrix for optimization of the PG activity.

Run	X1 Temperature (°C)	X2 Time(d)	X3 pH	PG activity (U·mL^{-1})
1	−1(28)	−1(8)	0(4)	3.06 ±0.21
2	−1	1(12)	0	3.72 ±0.34
3	−1	0(10)	−1(3)	4.00 ±0.36
4	−1	0	1(5)	1.34 ±0.21
5	0(30)	−1	−1	4.41 ±0.24
6	0	1	−1	4.78 ±0.45
7	0	−1	1	2.67 ±0.39
8	0	1	1	4.48 ±0.58
9	1(32)	−1	0	1.18 ±0.41
10	1	1	0	1.39 ±0.38
11	1	0	−1	1.80 ±0.27
12	1	0	1	1.15 ±0.38
13	0	0	0	4.18 ±0.32
14	0	0	0	3.33 ±0.17
15	0	0	0	3.60 ±0.31
16	0	0	0	4.12 ±0.64
17	0	0	0	3.56 ±0.33

isolates have significantly different hydrolysis ability, with $(D/d)^2$ values ranging from 1.43 ± 0.36 to 6.10 ± 0.13. The Congo red staining method is reliable because it avoids the identification of false positives, and it has been used for decades.[26] The Gyx086 strain exhibited the biggest $(D/d)^2$ value (6.10 ± 0.13), which suggested that this strain possessed the highest hydrolysis ability. The *A. niger* Gyx086 was therefore preliminarily selected for the next part of the experiment.

Effect of temperature, pH and inoculum size on pectinase activity

Pectinase combined with other enzyme has been used widely used for improving the production of fruit juice in the extraction and clarification process.[27,28] In this study, the Gyx086 strain was inoculated into the date residues medium for pectinase' enzyme cocktail production. The effects of temperature, pH value, and inoculum size on pectinase activity were investigated and the results are shown in Fig. 1. Visually, the *A. niger* grew well from pH 3 to pH 7 and a distinct suppression raised at pH 9.0, consistent with pectinase activities in Fig. 1(a). It suggested that pectinase secretion was greatly affected by the pH: pH 3 to 5 were more suitable for producing higher activity from the pectinase enzymes than other pH condition, which was consistent with previous research.[16] As Fig. 1(b) shows, the most suitable

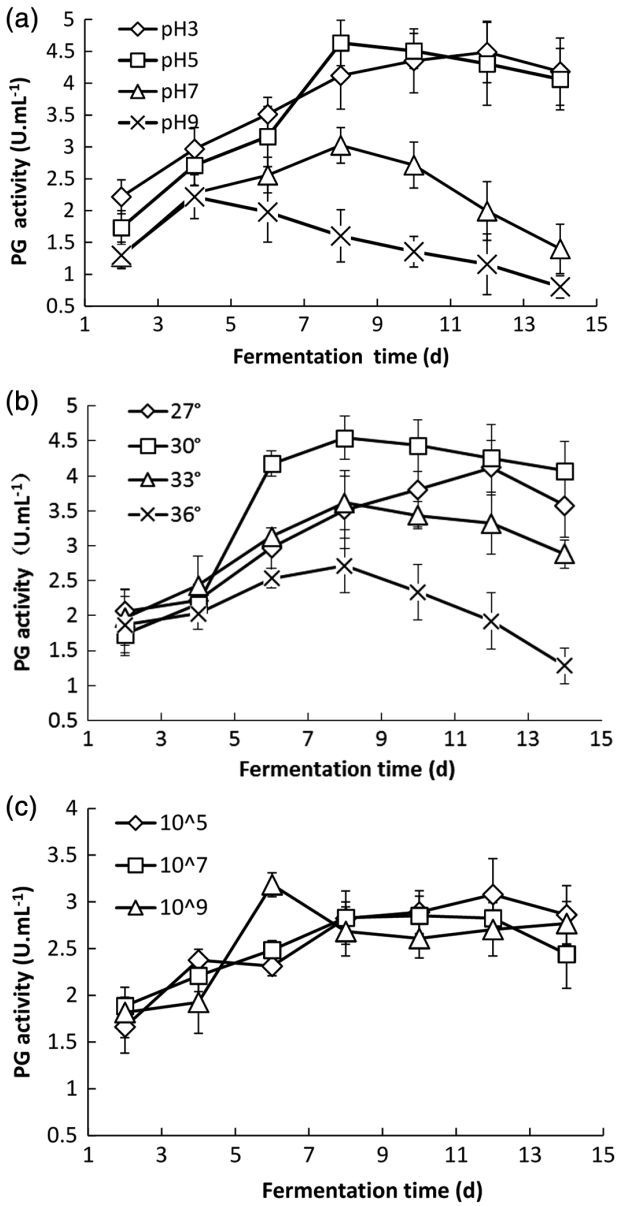

Figure 1. Effect of pH (a), temperature (b) and inoculum size (c) on pectinase activity.

temperature was 30 °C for high pectinase activity, and when the temperature was higher or lower than this, pectinase activity would be significantly reduced ($\alpha = 0.05$). Moreover, the fermentation time distinctly affected pectinase activity in Fig. 1, and 8 days' fermentation was usually needed to obtain relatively higher pectinase activity, but the activity was subsequently reduced. However, inoculum sizes from 10^{-5} to 10^{-9} in 50 mL medium with a constant 5% of date residues have not significantly affected pectinase activity during fermentation. Based on the above results, more suitable levels of temperature, pH values, and fermentation time

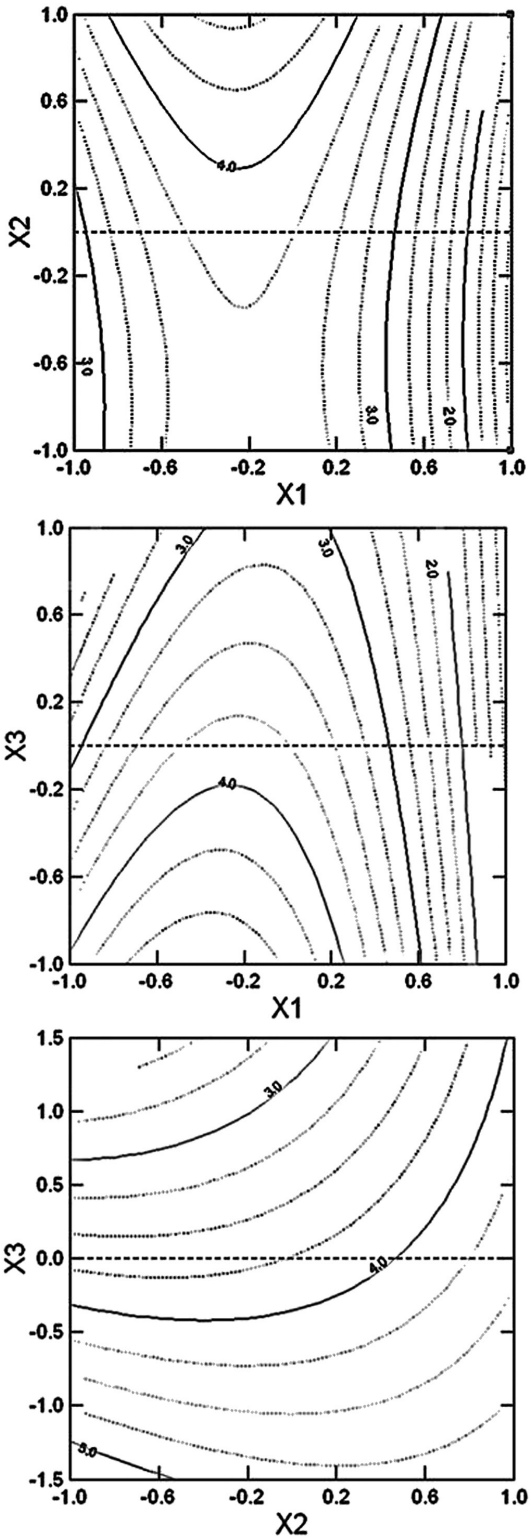

Figure 2. 2-D contour plots on interactive effects among temperature(X1), fermentation time(X2) and pH(X3) on PG activity.

were selected for the Box–Behnken design shown in Table 2, and the inoculum size was decided at 10^{-7} in each flask with 50 mL medium containing a constant 5% date residue.

Optimization of PG production

The optimized conditions for PG production can be evaluated by response surface analysis (RSA).[29] The experimental runs based on the BBD design and the experiment results are presented in Table 2. Fermentation temperature (X1), time (X2), and pH value (X3) were the independent variables and PG activity was the response variable. Seventeen tests were conducted and a complete quadratic model could be built between response and independent variables by regression analysis. The complete quadratic model could be expressed with the following quadratic equation:

$$PGA\left(\mathrm{U}.ml^{-1}\right) = 3.758 - 0.827\mathrm{X1} + 0.382\mathrm{X2} - 0.668\mathrm{X3} - 1.718\,\mathrm{X1}^2 + 0.294\,\mathrm{X2}^2 + 0.032\,\mathrm{X3}^2 - 0.113\,\mathrm{X1X2} + 0.360\mathrm{X2X3} + 0.503\mathrm{X1X3}$$

The results of the variance analysis for the model are shown in Table 3. The squared multiple *R* is 0.961, which indicates that the model can explain the test results well.[30] The *P* values of 'regression' and 'lack of fit' were 0.001 (< 0.05) and 0.260 (> 0.05), respectively, which indicated that the model is credible for assessing PG activity.[16,30] All of 2-D contour plots are presented as non-circle graphs (Fig. 2), which suggest that the temperature, pH, and fermenting time interactively affected the PG activity

Table 3. The variance analysis ANOVA and the lack-of-fit test for the response surface quadratic model.

Source	df	Type I SS	Mean squares	F ratio	*P* value
Regression	9	24.398	2.711	13.765	0.001
Linear	3	10.207	3.402	17.275	0.001
Quadratic	3	12.612	4.204	21.347	0.001
Interaction	3	1.579	0.526	2.672	0.128
Residual error	7	1.379	0.197		
Total error	16	25.776			
Squared multiple R	0.947				
Adjusted squared	0.878				
Lack of fit	3	0.823	0.274	1.976	0.260
Pure error	4	0.555	0.139		
Residual error	7	1.379	0.197		

during fermentation.[22] However, the most optimized value was a saddle point. A ridge analysis was therefore conducted using SASTAT 12 software (Systat Software, Inc., USA). In the range of experimental conditions, the optimal forecasted response is 4.674 (95.00% confidence interval from 4.09 to 5.26). The most optimized condition of X1, X2 and X3 were −0.305, 0.415, and − 0.857, respectively, corresponding to temperature 29.39 °C, time 10.83 days, and pH 3.143, respectively. For confirmation of the optimal forecast value, five batches of the fermentation trials were executed at 29.5 °C for 11 days at an initial pH 3.1. These simplified conditions are close to the optimal values, which are considered to be the controllable parameters when producing juice in an industrial fermentation tank for the industrial scale. Polygalacturonase activity was 4.79 ± 0.35 U.mL^{-1}, which is near to the forecast value. As a result, the optimized conditions were used for the production of an enzyme cocktail with high PG activity. Furthermore, xylanase and CMCase activity were 3.78 ± 0.31 U.mL^{-1} and 0.29 ± 0.11 U.mL^{-1}, respectively, in this enzyme cocktail. The synergistic effect with various lignocellulase within the enzyme cocktail could be more effective in degrading the cell wall,[17] thus improve the juice releasing from the date cell.

Enzymatic treatments in fruit juices of Muzao date

To use PG for the enzymatic hydrolysis of Muzao dates to improve juice production, the characteristics of temperature, pH, and heat resistance were investigated. The PG displays the highest enzymatic activity at 40 °C, with the reaction at pH 5.0, which is no different from the PG using wheat straw as fermentation material.[16] Under these conditions, PG has a good thermostability without a significant loss of enzymatic activity during 12 h of hydrolysis test. It is thus suitable for the production of Muzao date juice. The solid-to-liquid ratio, hydrolysis time, and enzyme cocktail content were further investigated. As shown in Fig. 3, the juice yield was significantly increased with solid-to-liquid ratios from 1:10 to 1:28 when only water was used as the extraction solvent. However, higher yield usually occurs with lower soluble solid content (SSC) such as 61.8% juice yield with 7.45 ± 0.02% SSC at a 1:10 rate, but 88.2% juice yield with only 2.53 ± 0.02% SSC at 1:28, which was difficult to use for beverage material due to too the SSC being too low. The rate of 1:16 was therefore used for further trials due to its appropriate SSC for producing fruit juice. When the PG was used to enhance the extraction of Muzao juice, the juice yield could

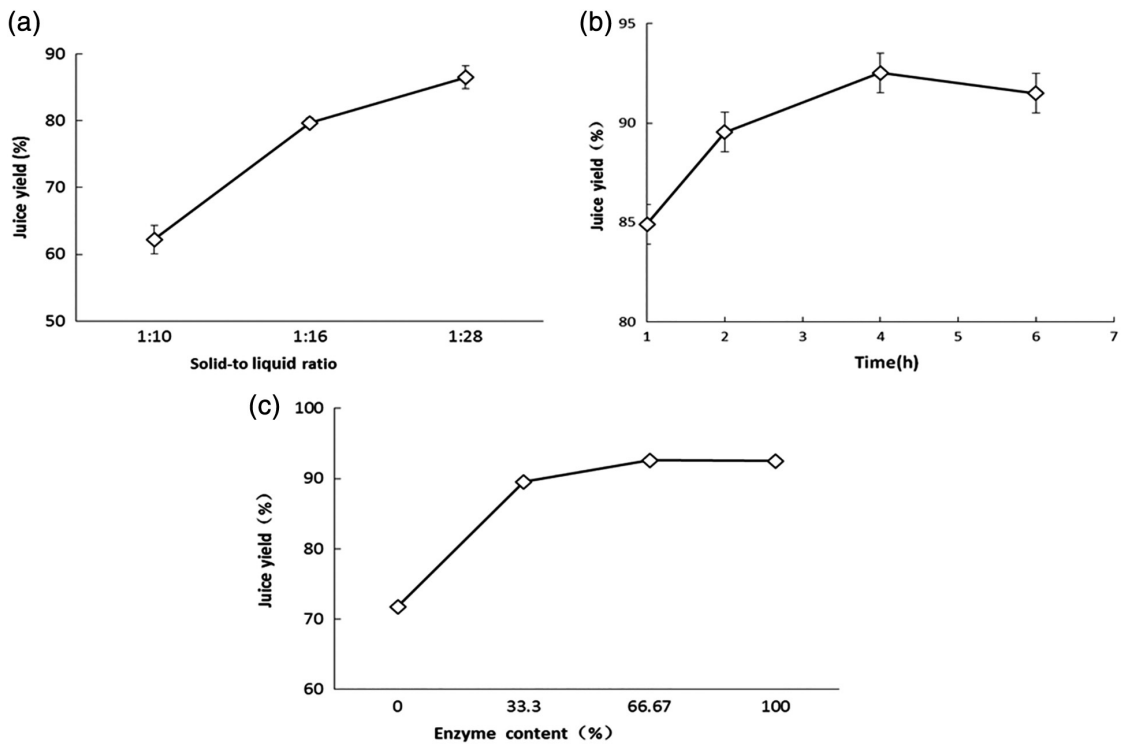

Figure 3. Effect of solid to liquid (a), hydrolysis time (b) and enzyme cocktail content (c) on the juice yield of Muzao date.

be significantly increased to 92.4% after 4 h of enzymatic hydrolysis. A longer hydrolysis time would not be beneficial in increasing the yield, as shown in Fig. 3(b). Moreover, 33.3% of enzyme broth content is enough for assisting the juice extraction and higher content of enzyme cocktail could not improve the juice yield (Fig. 3(c)).

Optimizing the yield of Muzao date juice

Base on the above results, another optimized process was demonstrated using an RSA method with hydrolysis time (X1), solid to liquid rate (X2), and enzyme cocktail content (X3) as factors, and the juice yield as the response variable. The BBD design and the experiment results are shown in Table 4. The juice yield was expressed using a complete quadratic model as the following quadratic equation by regression analysis:

$$\text{juice yield}(\%) = 91.840 + 0.324X1 + 0.754X2 - 0.101X3 - 0.039X1^2 + 0.246\,X2^2 - 0.067\,X3^2 - 0.304\,X1X2 + 0.008X2X3 - 0.449X1X3$$

The results of ANOVA for this model are shown in Table 5. The squared multiple R is 0.849, which indicates that the model can explain the test results well.[30] The *P* values of 'regression' and 'lack of fit' were 0.032 and 0.592, respectively, which indicated that the model is credible.[16,30] All 2-D contour plots present as non-circle graphs in Fig. 4, which suggests that the hydrolysis time, solid-to-liquid ratio, and enzyme cocktail content interactively affected the juice yield.[31] The most optimized value was a quiescent point with a juice yield of 92.844% (95.00% confidence interval from 92.25% to 93.44%) analyzed by SASTAT 12 software (Systat Software, Inc., USA). The most optimized conditions of X1, X2, and X3 are −0.044, 0.996, and − 0.082, respectively, corresponding to a hydrolysis time of 3.54 h, a solid-to-liquid ratio of 1:17.99 and enzyme cocktail content of 53.77%, respectively. To confirm the optimal forecast value, five batches of the fermentation trials were executed at 40 °C at pH 5.0, solid-to-liquid ratio 1:18, an enzyme cocktail content of 54%, and enzymatic hydrolysis pretreatment for 3.5 h. These conditions are close to the optimal values and considered as achievable and controllable when yielding Muzao date juice at an industrial scale, which is generally carried out in a sealed industry hydrolyzation tank. As a result, the juice yield was 92.88 ± 0.75%, which is very near to the forecast value. This demonstrated that the quadratic equation can be used effectively to evaluate the juice yield from the enzymatic hydrolysis of Muzao dates.

Table 4. Box–Behnken design matrix for optimization of the juice yield.

Run	X1 Time (h)	X2 Solid: Liquid ratio (g:mL)	X3 Enzyme cocktail content (%)	Juice yield (%)
1	−1(2.5)	−1(1:14)	0(55)	90.64 ± 0.51
2	−1	1(1:18)	0	92.47 ± 0
3	−1	0(1:16)	−1(30)	91.31 ± 0.31
4	−1	0	1(70)	91.85 ± 0.32
5	0(3.5)	−1	−1	91.16 ± 0.09
6	0	1	−1	92.93 ± 0.33
7	0	−1	1	91.09 ± 0.42
8	0	1	1	92.9 ± 0.77
9	1(4.5)	−1	0	92.23 ± 0.48
10	1	1	0	92.84 ± 0.29
11	1	0	−1	92.52 ± 0.36
12	1	0	1	91.27 ± 0.74
13	0	0	0	91.1 ± 0.88
14	0	0	0	92.04 ± 0.38
15	0	0	0	91.94 ± 0.35
16	0	0	0	92.28 ± 0.87
17	0	0	0	91.84 ± 0.31

Table 5. Response surface ANOVA of the quadratic model of the date juice yield.

Source	df	Type I SS	Mean squares	F ratio	*P* value
Regression	9	6.912	2.524	4.377	0.032
Linear	3	5.467	2.765	10.386	0.006
Quadratic	3	0.270	4.675	0.514	0.686
Interaction	3	1.175	0.132	2.233	0.172
Residual error	7	1.228	1.904		
Total error	16	8.140			
Squared multiple R	0.849				
Adjusted squared	0.655				
Lack of fit	3	0.429	0.143	0.716	0.592
Pure error	4	0.799	0.200		
Residual error	7	1.228	0.175		

Characteristics of date juice

To investigate the characteristics of date juice produced by the experimental enzymatic preparations, a non-enzymatic extraction was used for the control and a commercial enzyme preparation extraction was used for

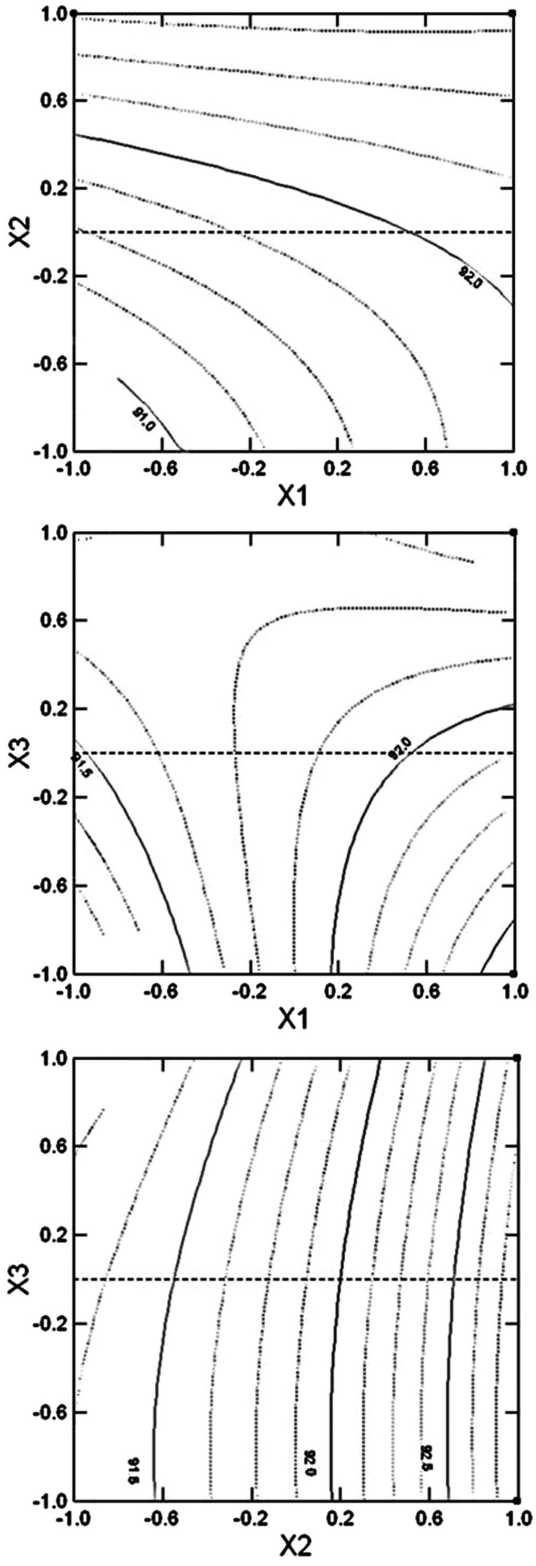

Figure 4. 2-D contour plots regarding the interactive effects among hydrolysis time (X1), solid-to liquid ratio (X2), and enzyme cocktail content (X3) on jujube juice yield.

the contrast. Enzymatic treatment greatly improved the juice yield. Water extraction without any enzyme gave a yield of 83.65 ± 0.42% juice yield whereas the enzyme-assisted extraction could obtain 92% of juice yield with 92.88 ± 0.75% for the experimental preparation and 92.11 ± 0.78% for the commercial preparation. It is for this reason that the pectinase, usually including cellulase and hemicellulase,[16,32] could degrade the lignocellulose in the cell wall to facilitate the release of juice from the cell. Clarification, viscosity, flavone content, and the degree of browning, were assayed in the date juices and the results are shown in Fig. 5. Enzyme-treated juices are much clearer than non-enzymatic extractions and there is a slower reduction of clarity (Fig. 5(a)). Similar results have been manifested in grape[33] and cupuacu.[34] Pectin is mainly responsible for turbidity and for causing an increase in viscosity,[31] so pectinase, which hydrolyzes pectin to small, soluble molecules, plays a crucial role in fruit juice production. As a result, juice viscosity also declined significantly (Fig. 5(b)) and the experimental preparation had the best effect on the date juice viscosity. The stability of viscosity in the enzyme treatment date juice was better than that in the non-enzyme treatment juice, and many precipitates appeared in the latter during storage. However, the enzyme-treated juice possessed lower flavone content than non-enzyme-treated juice. The latter therefore has a lower degree of browning in the early stages of storage, as shown in Fig. 5(d). The soluble solid content was also determined in these juices. The enzyme-treated juices including the experimental preparation and the commercial preparation have a higher soluble solid content, at 4.0% and 4.5%, respectively, than the non-enzyme treated juice, which indicates that enzyme treatment enhances the quality of soluble solids in date juice.

Conclusion

Gyx086 is a potential candidate for producing high activity pectinase which can reach 4.79 ± 0.35 $U.mL^{-1}$ using low-cost date residues as the medium. The date-juice yield could be significantly enhanced to 92.88% under the optimized conditions used in the experiment. This was 11% higher than the control. The experimental preparation could replace the expensive commercial preparation to greatly cut down production costs and improve the quality of date juice in terms of clarity, viscosity, storage stability, and soluble solid content. This is the first systematic report on Muzao date juice production achieved by a cheap and effective pectinase cocktail.

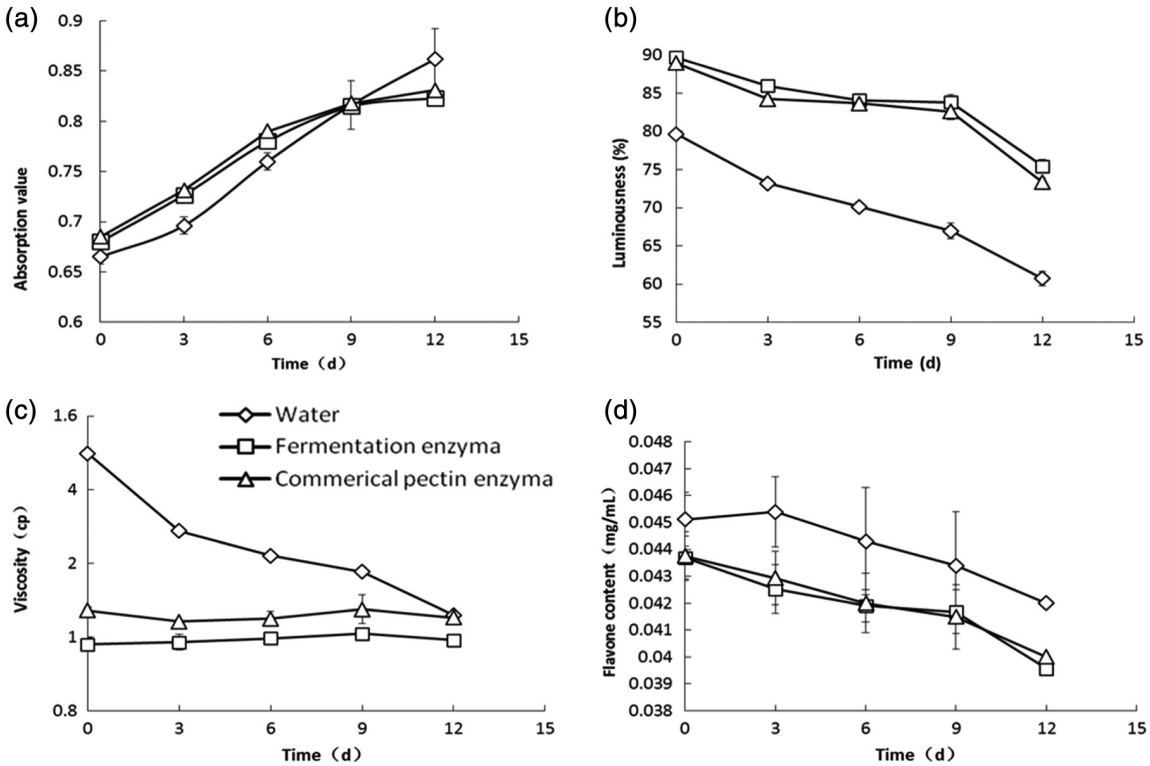

Figure 5. Change of date juice yield during storage periods: (a) luminousness; (b) viscosity; (c) flavone content; (d) degree of browning.

Acknowledgements

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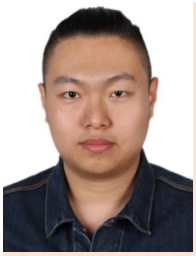

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