Original article

Optimization of processing parameters for natural cloudy mango (*Mangifera indica* L.) juice using pectolytic and cellulolytic enzymes

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Optimization of processing parameters for natural cloudy mango (*Mangifera indica* L.) juice using pectolytic and cellulolytic enzymes.

Abstract — **Introduction**. Many desirable properties make cloudy fruit juice widely produced according to consumer acceptance. The pulp of mango (*Mangifera indica* L.), a good source of carbohydrates and water, has many uses including the production of juice. Nowadays, the use of food enzymes has become widespread in the juice industry for various reasons. However, the application of enzymes has not yet been tested in the context of the production of cloudy mango juice, except for the stabilization of nectars. Thus, the aim of our study was to examine and optimize some parameters involved in the enzymatic production of cloudy mango juice. **Materials and methods**. To achieve our objective, we used Response Surface Methodology (RSM) by combining five factors: time (30–50 min), temperature (35–55 °C), pH 4–5.5, Pectinex® Ultra SP-L (20–40 $\mu L \cdot L^{-1}$) and Cellubrix® L (15–25 $\mu L \cdot L^{-1}$). **Results and discussion**. The study of juice yield, cloud stability and the browning index showed that the coefficients of determination (R^2 values) of these traits with the studied parameters were greater than 0.900. The optimized juice yield, cloud stability and browning index varied with specific values chosen for the five parameters mentioned above. **Conclusions**. Finally, the use of 30 $\mu L \cdot L^{-1}$ Pectinex® Ultra SP-L and 22 $\mu L \cdot L^{-1}$ Cellubrix at 45 °C, for 43 min, at pH 5.5, was found to be the optimum set of conditions for processing cloudy mango juice.

China / *Mangifera indica* / fruit juices / processing / colloidal properties / stabilizers / glycosidases / statistical methods

Optimisation des paramètres pour le traitement de jus de mangue (*Mangifera indica* L.) naturellement trouble avec l'emploi d'enzymes pectolytiques et cellulolytiques.

Résumé — **Introduction**. De nombreuses caractéristiques intéressantes ont permis de produire largement des jus de fruits troubles conformes avec l'acceptabilité du consommateur. La pulpe de mangue (*Mangifera indica* L.), une bonne source d'hydrates de carbone et d'eau, a de nombreuses utilisations dont la production de jus. Aujourd'hui, l'utilisation d'enzymes agroalimentaires est devenue très répandue dans les industries du jus pour diverses raisons. Cependant, l'application d'enzymes n'a pas été encore testée dans le contexte de la production de jus de mangue trouble, si ce n'est pour la stabilisation de nectars. De ce fait, l'objectif de notre étude a été d'étudier, pour optimisation, certains paramètres impliqués dans la production enzymatique de jus de mangue trouble. **Matériel et méthodes**. Pour atteindre notre objectif, nous avons utilisé la méthodologie de surface des réponses en combinant cinq paramètres : le temps (30–50 min), la température (35–55 °C), le pH (4–5,5), et des enzymes tels que Pectinex® Ultra SP-L (20–40 $\mu L \cdot L^{-1}$) et Cellubrix® L (15–25 $\mu L \cdot L^{-1}$). **Résultats et discussion**. L'étude du rendement en jus, de la stabilité du trouble et de l'index de la coloration brune ont montré que les coefficients de corrélations (valeurs de R^2) de ces caractères avec les paramètres étudiés étaient tous supérieurs à 0,900. Le rendement en jus, la stabilité du trouble et l'index de la coloration brune optimisés ont varié en fonction des valeurs spécifiques choisies pour les cinq paramètres cités précédemment. **Conclusions**. Finalement, l'utilisation de 30 $\mu L \cdot L^{-1}$ de Pectinex® Ultra SP-L, de 22 $\mu L \cdot L^{-1}$ de Cellubrix® L à 45 °C, pendant 43 min et à un pH de 5,5, a été retenue comme condition optimale pour le traitement du jus de mangue trouble.

Chine / *Mangifera indica* / jus de fruits / traitement / propriété colloïdale / agent de texture / glycosidase / méthode statistique

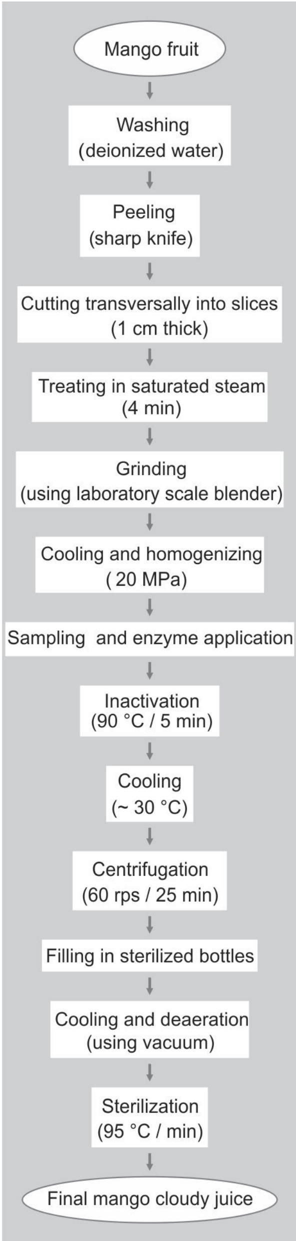


Figure 1.
Flow chart of cloudy mango juice production.

1. Introduction

Mango fruit pulp, 33–85% of the fresh fruit, is essentially composed of carbohydrate (16.20–17.18 g), protein (0.36–0.40 g), carotene (0.135–1.872 mg), pectin, polyphenols, minerals and other vitamins. Water content represents the most significant part (more than 80%) [1, 2].

Cloudy fruit juices contain colloidal suspensions which cause turbidity, carrying flavor substances and natural antioxidants such as carotenoids, polyphenols, etc. [3]. In fact, to modify physical-chemical properties in some way (flavor, texture, appearance or storage stability of foods), enzymes have been used to produce fruit juice and vegetable juice (processing aids). Most of the enzymes' applications to fruits and vegetables required the destruction of tissue integrity by promoting the added enzyme and its substrate (pulp) to be in contact [4]. Improving the quality of mango juice can be accomplished by treating the mango pulp with liquefying enzymes, resulting in lower viscosities and high juice yield [5]. Cloudy fruit juices are becoming more and more popular, whose suspension stability, color and flavor represent key elements for consumer acceptance. In fact, cloud stability improvement is another quality of enzyme application [6].

To date, most research done on mango pulp can be summarized as: enzymatic liquefaction before concentration by reverse osmosis [7] and improving the cloud stability of mango nectars by using enzyme mixtures of polygalacturonase, pectinlyase, cellulases and hemicellulases [8]. In addition, the degradation of quality attributes of two varieties of mango juice during storage has also been investigated. No enzyme was used and water was added at the ratio of 1:4 [9].

However, to date no report has been published on the production of natural cloudy mango juice using enzymes (pectinases, cellulases, or both). Therefore, the overall objective of the present work was to study the effect of enzyme treatment (Pectinex® Ultra SP-L dosage and Cellubrix® L dosage, hydrolysis time, temperature and pH) on cloudy mango juice and to optimize the process conditions by using response surface methodology (RSM).

2. Materials and methods

2.1. Materials

2.1.1. Experimental material

Fresh and completely ripe mangoes (*Mangifera indica* L.) were obtained from a supermarket, in Wuxi Jiangsu province, China.

2.1.2. Enzymes used

Pectinex® Ultra SP-L (mainly polygalacturonase) from *Aspergillus aculeatus* and Cellubrix® L (cellulase) from *Trichoderma reesei* were obtained from Novozymes Investment Co. Ltd. (Beijing, China). The choice of these two enzymes was essentially based on the pre-test results (not reported). The enzyme activities are 9500 polygalacturonase (PGU) units·mL^{-1} for Pectinex® Ultra SP-L and 700 endoglucanase (EGU) units·g^{-1} for Cellubrix® L.

All other chemicals were analytical-grade products obtained commercially.

2.2. Methods

2.2.1. Cloudy mango juice preparation

Cloudy mango juice was prepared according to a specific process (*figure 1*). Many enzymes such as pectinase, hemicellulase (from Hunan New Century Biochemical Co., Ltd. in Hunan Province, P.R.C), Rapidase TF, Rapidase FP super (from DSM Food Specialties - Beverage Ingredients, Delft, The Netherlands), Pectinex Yield Mash, and Pectinex Smash XXL, Pectinex® Ultra SP-L and Cellubrix® L (from Novozymes, Beijing, China) were used and even combined. Pectinex® Ultra SP-L and Cellubrix® L were selected according to the results obtained after some tests on yield, stability and cloudiness (results not reported).

Based on preliminary experiments, five treatments were used during enzymatic reaction: Pectinex® Ultra SP-L (20–40 μL·L^{-1}), X1; Cellubrix® L (15–25 μL·L^{-1}), X_2; incubation time (30–50 min), X_3; temperature (35–55 °C), X_4; and pH (4–5.5), X_5. NaOH (1 N) and HCL (1 N) were used to adjust the pH. The rotation was fixed (300 r·min^{-1})

using an IKA® RW 20 digital (US) overhead stirrer package and enzymes were inactivated by heating the suspension in a water bath at 90 °C for 5 min.

2.2.2. Juice yield

The juice yield was calculated using the following formula: juice yield = [(m /m_i) × 100], where m is the mass of the expressed juice and m_i is the initial mass of mango pulp.

2.2.3. Cloud stability

The cloud stability was deduced from the relative turbidity (T %): T = [(T_c / T_0) × 100], where T_0 and T_c are the juice turbidities before and after centrifugation at 4200 g for 15 min (ambient temperature), respectively [10].

2.2.4. Browning index

The Browning Index (BI), which represents the purity of brown color, is an important parameter in processes where enzymatic or non-enzymatic browning takes place. It was calculated as described by Palou *et al.* [11]. Hunter a (redness value), b (yellowness value) and L (lightness value) parameters of different mango juice samples were determined with a Konica Minolta Chroma meter (CR-400; Konica Minolta, Tokyo, Japan). The instrument was standardized with a white ceramic plate (a = 0.14, b = 1.64, L = 97). The BI was calculated according to the following equations:

$$BI = \frac{100\ (x - 0.31)}{0.172},$$

$$\text{where } x = \frac{(a + 1.75)}{(5.645\ L + a - 3.012\ b)}$$

2.2.5. Experimental design and statistical analysis

Response Surface Methodology (RSM) was used in designing this experiment. Statistical Analysis System (SAS Institute Inc., Cary, NC, USA) Software 8.1 (2000) was applied. Five independent variables: Pectinex® Ultra SP-L (x_1), Cellubrix® L (x_2), hydrolysis time (x_3), temperature (x_4) and pH (x_5) were chosen, with X the actual variable. Each independent variable had five levels which were – 2, – 1, 0, + 1 and + 2. A total of 32 different combinations were chosen in random order according to Central Composite Uniform Precision (CCUP) (*table I*).

The responses measured include juice yield (y_1), cloud stability (y_2) and the browning index (y_3) (*table II*). These values were related to the coded variables (x_i, with i = 1, 2, 3, 4 and 5) by a second-degree polynomial using the equation:

$$y_i = f(X_1, X_2, X_3, X_4, X_5)$$

$$y_i = b_0 + \sum_{i=1}^{5} b_i x_i + \sum_{i=1}^{5} b_i^2 x_i^2 + \sum_{i=1}^{4} \sum_{j=i+1}^{5} b_{ij} x_{ij} \quad \text{(equation 1)}$$

The coefficients of the polynomial were represented by b_0 (constant term), b_i (linear effects), b_i^2 (quadratic effects), and b_{ij} (interaction effects). Each treatment was subjected to analysis of variance (ANOVA) to determine the effects of independent variables as well as their interactions on the responses. The significances of all terms in the polynomial were judged statistically by calculating the F-value at a probability (p) of 0.001, 0.01 or 0.05.

3. Results and discussion

The effect of the independent variables (Pectinex® Ultra SP-L, Cellubrix® L, incubation time, temperature and pH) on the three response functions (juice yield, cloud stability and the browning index) was obtained through the experimental results (*table II*). The importance of the coefficient of determination in the models is defined as R^2, which expresses the degree of fitness of the proposed model and the empirical one. It also depicts the proportion of the variability in the response variable, which is accounted for by the regression analysis [12]. The closer the value R^2 is to unit, the better the empirical model fits the actual data.

Analysis of variance for the three response functions (*table III*) indicated that the response variables were adequate. The R^2 values for juice yield, cloud stability and the browning index were 0.984, 0.994 and 0.981, respectively, indicating that the regression models expound the reaction well. The probability (p) values of all

Table I.
The central composite uniform precision (in coded levels of five variables) used for enzymatic cloudy mango juice extraction.

Serial number	Pectinex® Ultra SP-L (µL·L^{-1}) X_1 (x_1)	Cellubrix® L (µL·L^{-1}) X_2 (x_2)	Incubation time (min) X_3 (x_3)	Temperature (°C) X_4 (x_4)	pH X_5 (x_5)
1	20 (– 1)	15 (– 1)	30 (– 1)	35 (– 1)	5.5 (+ 1)
2	20 (– 1)	15 (– 1)	30 (– 1)	55 (+ 1)	4 (– 1)
3	20 (– 1)	15(– 1)	50 (+ 1)	35 (– 1)	4 (– 1)
4	20 (– 1)	15(– 1)	50 (+ 1)	55 (+ 1)	5.5 (+ 1)
5	20 (– 1)	25 (+ 1)	30 (– 1)	35 (– 1)	4 (– 1)
6	20 (– 1)	25 (+ 1)	30 (– 1)	55 (+ 1)	5.5 (+ 1)
7	20 (– 1)	25 (+ 1)	50 (+ 1)	35 (– 1)	5.5 (+ 1)
8	20 (– 1)	25 (+ 1)	50 (+ 1)	55 (+ 1)	4 (– 1)
9	40 (+ 1)	15 (– 1)	30 (– 1)	35 (– 1)	4 (– 1)
10	40 (+ 1)	15 (– 1)	30 (– 1)	55 (+ 1)	5.5 (+ 1)
11	40 (+ 1)	15 (– 1)	50 (+ 1)	35 (– 1)	5.5 (+ 1)
12	40 (+ 1)	15 (– 1)	50 (+ 1)	55 (+ 1)	4 (– 1)
13	40 (+ 1)	25 (+ 1)	30 (– 1)	35 (– 1)	5.5 (+ 1)
14	40 (+ 1)	25 (+ 1)	30 (– 1)	55 (+ 1)	4 (– 1)
15	40 (+ 1)	25(+ 1)	50 (+ 1)	35 (– 1)	4 (– 1)
16	40 (+ 1)	25 (+ 1)	50 (+ 1)	55 (+ 1)	5.5 (+ 1)
17	10 (– 2)	20 (0)	40 (0)	45 (+ 2)	4.75 (0)
18	50 (+ 2)	20 (0)	40 (0)	45 (0)	4.75 (0)
19	30 (0)	10 (– 2)	40 (0)	45 (0)	4.75 (0)
20	30 (0)	30 (+ 2)	40 (0)	45 (0)	4.75 (0)
21	30 (0)	20 (0)	20 (– 2)	45 (0)	4.75 (0)
22	30 (0)	20 (0)	60 (+ 2)	45 (0)	4.75 (0)
23	30 (0)	20 (0)	40 (0)	25 (– 2)	4.75 (0)
24	30 (0)	20(0)	40 (0)	65 (+ 2)	4.75 (0)
25	30 (0)	20 (0)	40 (0)	45 (0)	3.25 (– 2)
26	30 (0)	20 (0)	40 (0)	45 (0)	6.25 (+ 2)
27	30 (0)	20 (0)	40 (0)	45 (0)	4.75 (0)
28	30 (0)	20 (0)	40 (0)	45 (0)	4.75 (0)
29	30 (0)	20 (0)	40 (0)	45 (0)	4.75 (0)
30	30(0)	20 (0)	40 (0)	45 (0)	4.75 (0)
31	30 (0)	20 (0)	40 (0)	45 (0)	4.75 (0)
32	30 (0)	20 (0)	40 (0)	45 (0)	4.75 (0)

regressions were less than 0.001, 0.01 in some cases, or less than 0.05 in other cases.

The best elucidative model equations fitted are given in equation 2 for juice yield, 3 for cloud stability, and 4 for the browning index:

$y_1 = 0.72\,x_1 + 1.91\,x_3 + 0.77\,x_4 + 1.33\,x_5 + 0.37\,x_2^2 - 1.51\,x_3^2 + 1.19\,x_3^2 + 0.66\,x_{13} + 0.53\,x_{15} + 0.57\,x_{23} - 0.53\,x_{24} + 1.68\,x_{45}$ (equation 2),

$y_2 = -1.82\,x_1 + 2.32\,x_3 - 1.03\,x_4 - 3.48\,x_1^2 - 4.59\,x_2^2 - 4.08\,x_3^2 - 0.82\,x_{12} - 2.35\,x_{14} + 5.99\,x_{15} - 4.24\,x_{24} - 4.66\,x_{34} - 0.96\,x_{45}$ (equation 3),

$y_3 = -0.84\,x_2 + 0.3\,x_3 - 0.50\,x_1^2 - 0.68\,x_3^2 - 0.38\,x_{12} - 1.89\,x_{13} - 1.12\,x_{14} - 1.51\,x_{23} - 0.78\,x_{24} - 0.44\,x_{34} - 0.86\,x_{35} + 0.68\,x_{45}$ (equation 4).

3.1. Juice yield

The response surfaces of various independent variables on juice yield are represented (*figure 2*). The juice yield was negatively related to the linear effects of Pectinex

Table II.
Effect of incubation time, temperature and enzymatic dosage on the three dependent variables used for studying enzymatic cloudy mango juice extraction.

Serial number	y_1 (yield) (%)	y_2 (cloud stability) (%)	y_3 (browning index) (BI)
1	62.0	40.65	19.08
2	62.5	71.00	22.13
3	63.8	64.70	28.39
4	69.0	53.18	30.14
5	63.0	60.01	22.93
6	65.3	53.22	27.25
7	64.0	63.00	24.43
8	63.7	60.01	25.59
9	62.6	38.70	25.80
10	67.9	63.09	28.22
11	65.6	70.20	25.24
12	65.0	51.24	25.59
13	64.0	62.40	27.97
14	59.0	42.02	23.02
15	68.1	63.00	23.30
16	73.0	49.43	17.87
17	63.6	59.10	23.68
18	66.3	50.10	23.68
19	66.0	50.46	28.41
20	67.0	49.87	24.45
21	54.0	49.21	22.21
22	64.0	55.13	23.71
23	63.6	69.85	25.74
24	66.7	67.13	26.50
25	67.6	65.70	26.27
26	72.0	68.40	26.10
27	64.3	69.16	25.94
28	65.3	69.82	26.11
29	64.7	68.09	24.04
30	64.9	69.10	25.57
31	65.0	69.60	25.93
32	65.1	68.81	25.84

concentration ($P < 0.001$), temperature ($P < 0.001$) and pH ($P < 0.001$), and positively related to incubation time ($P < 0.001$). The interaction effects between Pectinex concentration and time ($P < 0.01$), Pectinex and pH ($P < 0.05$), and temperature and pH ($P < 0.01$) were positive, whereas the interaction effect between Cellubrix concentration and temperature ($P < 0.05$) was negative (*table III*).

The dependence of yield on pH and Pectinex concentration at fixed Cellubrix concentration, hydrolysis time and temperature was clear (*figure 2a*). The juice yield decreases as pH increases, up to around pH 4.5, and then gradually rises with increase in Pectinex concentration. As, we know the pH range of Pectinex® Ultra SP-L is wide and involves the active side of the enzyme. Also, the pH is extremely linked with the enzyme's activity and temperature. Hence, this result observed.

The reliance of juice yield on temperature and Cellubrix concentration is also described (*figure 2b*). In fact, at temperatures lower than 45 °C (with other factors at

Table III.
ANOVA effects of regression coefficients on the three dependent variables used for studying enzymatic cloudy mango juice extraction.

Regression coefficient	Juice yield (%)	Cloud stability (%)	Browning index
Linear effect coefficient			
b_0	146.8582	– 247.417	– 71.9034
b_1	– 0.5357 ***	– 0.7880 ***	1.8838
b_2	– 0.8472	11.940	1.7755 ***
b_3	0.8441 ***	6.0130 ***	2.4857 *
b_4	– 0.8974 ***	4.7774 **	0.3102
b_5	– 30.8623 ***	– 8.3090	– 2.0104
Quadratic effect coefficient			
b_1^2	– 0.0002 ***	– 0.0348 ***	– 0.0050 ***
b_2^2	0.0147 *	– 0.1837 ***	0.0075
b_3^2	– 0.0151 ***	– 0.0409 ***	– 0.0068 ***
b_4^2	0.0003	– 0.0001	0.0011
b_5^2	2.1216 ***	– 0.6586	0.2269
Interaction effect coefficient			
b_{12}	0.0054	– 0.0165 *	– 0.0164 ***
b_{13}	0.0065 **	0.0072 *	– 0.0188 ***
b_{14}	– 0.0019	– 0.0235 ***	– 0.0112 ***
b_{15}	0.0708 *	0.7993 ***	– 0.0023
b_{23}	0.0114 **	– 0.01	– 0.0301 ***
b_{24}	– 0.0106 *	– 0.0849 ***	– 0.0156 **
b_{25}	0.0317	0.024	0.0318
b_{34}	0.0038	– 0.0466 ***	– 0.0044 *
b_{35}	– 0.0092	– 0.0905	– 0.1152 ***
b_{45}	0.2242 **	– 0.1273*	0.0904 ***
R^2	0.9840	0.9939	0.9807

Subscripts: 1 = Pectinex® Ultra SP– L; 2 = Cellubrix® L; 3 = incubation time, 4 = temperature; 5 = pH.
***: P <0.001; **: P <0.01; *: P < 0.05.

their central points), juice yield increased with the rise in Cellubrix concentration. The same trend was adversely observed in a high temperature range (more than 45 °C).

In ground tissue, pectin (negatively charged) is found in the soluble phase, increasing viscosity and the pulp particles, whereas other pectin molecules remain bound to cellulose fibrils by means of side chains of hemicellulose and thus facilitate water retention [13]. During enzymatic treatment, Pectinex shatters the pectin molecules. Depending on the enzyme concentration, this degradation leads to a decrease in water-holding capacity [14], which in turn reduces the viscosity by slightly increasing the pH [15]. This was obvious during the centrifugation process, showing a liquefied juice at high enzyme concentration (more than 40 $\mu L \cdot L^{-1}$) in our experiments. As Urlaub describes pectin as heterogeneous mixtures of polysaccharides with different molecular weights and degrees of etherification [16], enzymatic degradation releases some soluble and non-soluble particles, making the juice cloudy in a range of enzyme concentration, pH and temperature, as we noticed. During this specific time, Cellubrix led to the degradation of cellulose and hemicellulose into sugars and insoluble particles, which increase the cloudiness, and consequently the juice yield (*figure 2*). As Karangwa *et al.* state, the technology advantage of pectic enzymes allows the improvement of juice yields [17]. This can be explained by additional released particles into the initial extracted juice. As we know, the pulpy state of mango pulp hampers juice extraction.

3.2. Cloud stability

Juices such as orange and tomato are usually cloudy and have colloidal suspensions. This cloudiness is desirable and acceptable to consumers [12]. According to Montenegro *et al.*, cloudy mango juice is also acceptable to consumers [3].

In finding an optimal cloudy mango juice, there were linear effects of Pectinex concentration ($P < 0.001$) on cloud stability, which was negative, while time ($P < 0.001$) and temperature ($P < 0.01$) were positively correlated (*table III*). The quadratic effects of Pectinex concentration, Cellubrix and hydrolysis time were all negative at $P < 0.001$. The interaction effects of Pectinex and Cellubrix concentration ($P < 0.05$), Pectinex and temperature ($P < 0.001$), Cellubrix and temperature ($P < 0.001$), hydrolysis time and temperature ($P < 0.001$), and temperature and pH ($P < 0.05$) were also all negative, whereas the interaction effects between Pectinex concentration and hydrolysis time ($P < 0.05$), and Pectinex and pH ($P < 0.001$) were positive. From the results, it can be seen that cloud stability varied linearly with Pectinex concentration, which is a source of released soluble particles during pectin hydrolysis. Also, temperature and hydrolysis time are important factors during cloudy mango juice production using enzymes. There was a curvilinear synergy effect between the two enzymes and cloud stability when the other factors were fixed at their central points (*figure 3a*). Based on this, both Pectinex and Cellubrix provide an optimum range for the highest cloud stability.

Based on the results, the response of cloud stability to temperature and Pectinex concentration is significant after fixing other factors at their central points (*figure 3b*). The effect of temperature and Cellubrix concentration on cloud stability was also noticeable (*figure 3c*). According to these two figures, cloud stability increased gradually with both Pectinex (mainly polygalacturonase) and Cellubrix concentrations at low temperatures, then decreased after reaching the optimum range. Kashyap *et al.* added exogenous enzymes with high levels of polygalacturonase activity to fruit juices to stabilize the cloud of citrus juices, purees and nectars [13].

From these two enzymes' effect in our study, the particle size distribution (analyzed by a Mastersizer, results not reported) showed two peaks with a peak average of 783.9 nm, which indicates that the final juice samples have relative cloud stability. Thus, from the literature, the best result for particle size reduction in lemon juice was obtained after enzymatic hydrolysis [18]. It can be

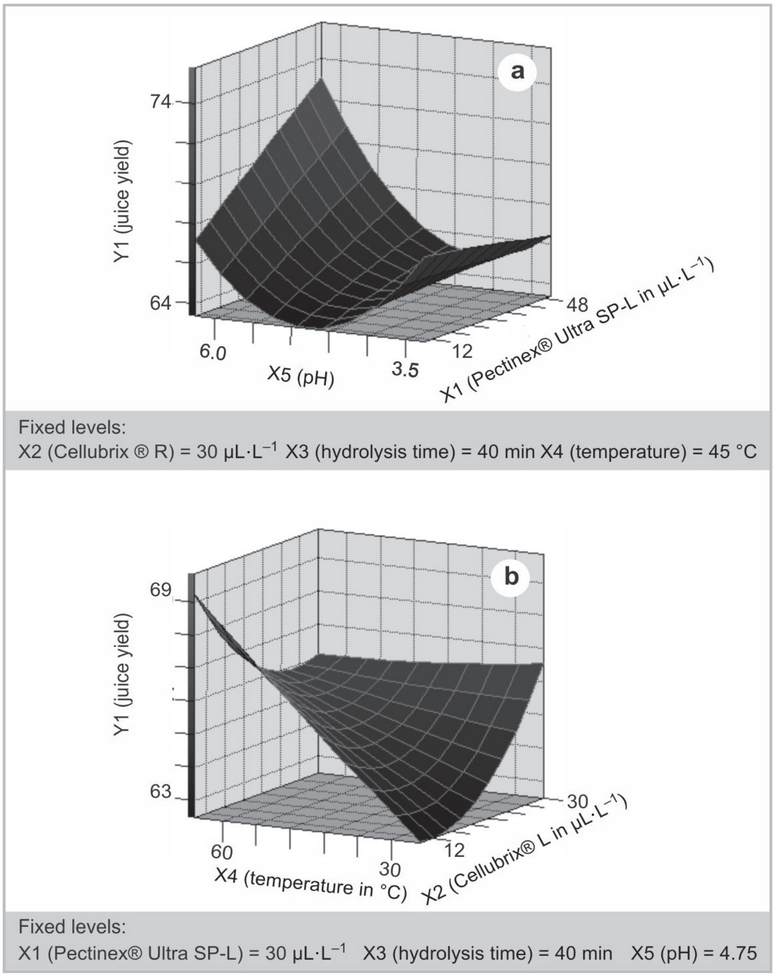


Figure 2.
Response surface for yield of cloudy mango juice as a function of (a) pH and Pectinex concentration, (b) temperature and Cellubrix concentration.

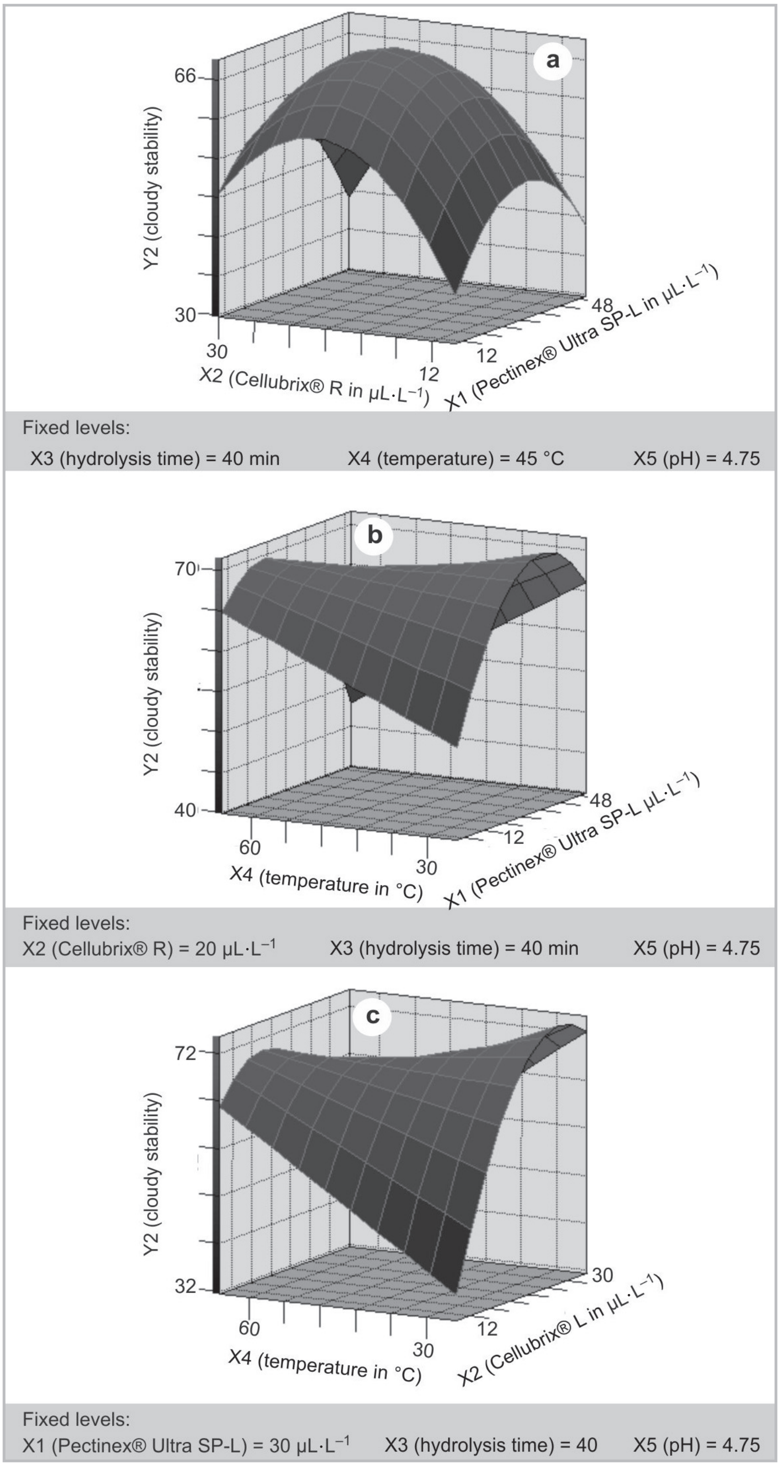
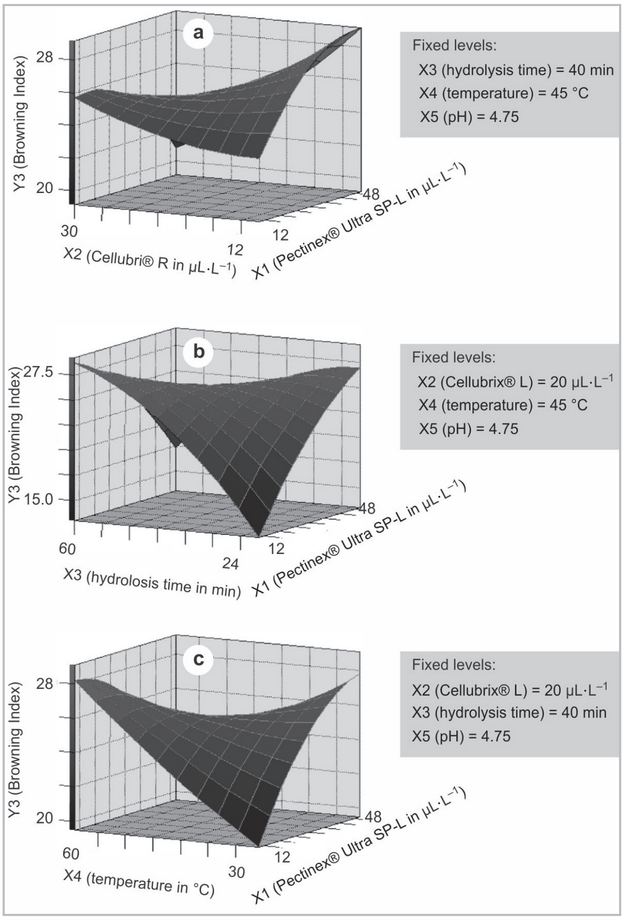


Figure 3.
Response surface for cloud stability of mango juice as a function of (a) Pectinex and Cellubrix concentrations, (b) Pectinex concentration and temperature, (c) Cellubrix concentration and temperature.

concluded that negatively correlated interaction effects led to cloudy juice, the samples being relatively stable. In addition, centrifugation speeds highlighted important effects on cloud stability (results not reported). The application of 60 rps (3600 rpm) centrifugation was found to produce the most stable juice in terms of cloudiness. As many cloudy juices (*e.g.*, apple, carrot, etc.) usually contain fine suspended particles, including cells, cell clusters, cell fragments and other macro-particles [19, 20], the results of this research can be linked to the report of Sin *et al.*, in which turbidity decreases with increasing enzyme concentration [12]. As such, cloud stability is directly linked to turbidity. From our results, at low enzyme concentrations, cloud stability increased gradually with temperature, attained an optimum level, and then decreased. When enzyme concentration was increased, pectin and other related carbohydrates were broken down, which induced a decrease in turbidity before and after centrifugation. The same result has been previously reported [21]; increase in enzyme concentration and incubation time may decrease the turbidity of the juice. In relation to degradation degree, turbidity is affected, as well as cloud stability from our results (*figures 3a*, *3b* and *3c*). Though the colloidal particles were small enough to be unaffected by gravity, within a few days sedimentation (Brownian motion) nonetheless occurred.

Based on this, it can be assumed that the use of natural specific gums is one way to increase the stability of cloudy mango juice; the adsorption of natural specific gum macromolecules at the surface of charged colloidal particles affects the structure of the electrical double layer and so increases the inter-particle repulsive potential. Another potential method to increase stability is to reduce the particle sizes by homogenization or milling. However, the mechanism of particle stabilization in cloudy juices using either of these two methods has not been completely described [19, 20], and as such no further assumptions can be made. According to Sorrivas *et al.*, many other interactions are involved in the mechanism of particle stabilization [22].



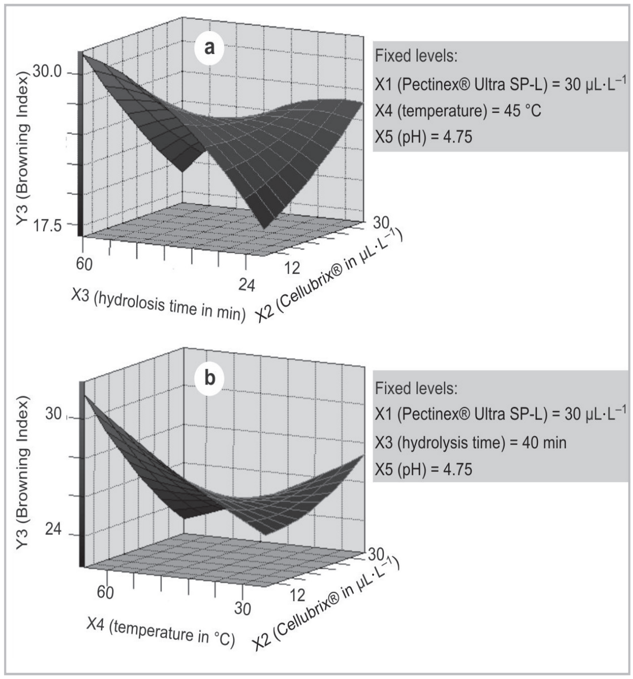


Figure 5.
Response surface for the browning index of mango juice as a function of (a) Cellubrix concentration and hydrolysis time, (b) Cellubrix concentration and temperature.

Figure 4.
Response surface for the browning index of mango juice as a function of (a) Pectinex and Cellubrix concentrations, (b) Pectinex concentration and hydrolysis time, (c) Pectinex concentration and temperature.

3.3. Browning index

The browning index (BI) has been defined as the purity of brown color [11]. Our results showed that the BI is positively correlated with the linear effect of Cellubrix concentration ($P < 0.001$) and incubation time ($P < 0.05$), while the quadratic effects of Pectinex concentration, Cellubrix and hydrolysis time were all negative at $P < 0.001$ (*table III*). Pectinex and Cellubrix concentration, Pectinex and hydrolysis time, Pectinex and temperature, Cellubrix and hydrolysis time, Cellubrix and temperature, and time and pH all presented negative interaction effects at $P < 0.001$, while temperature and pH interacted positively with the BI at $P < 0.001$. In addition, a negative interaction between hydrolysis time and temperature was also observed at $P < 0.05$ (*table III*).

The BI increased moderately at low enzyme concentrations before becoming quite constant at high concentrations (when other factors were kept at their central points) (*figure 4a*). At a constant Pectinex concentration, the BI gradually increased with hydrolysis time (*figure 4b*). The same trend is observed with temperature (*figure 4c*).

Regarding hydrolysis time, at a fixed Cellubrix concentration, the BI increased rapidly before decreasing when an optimum range time of 36–45 min was reached (*figure 5a*). Regarding temperature, a decrease in the BI was observed when the range of

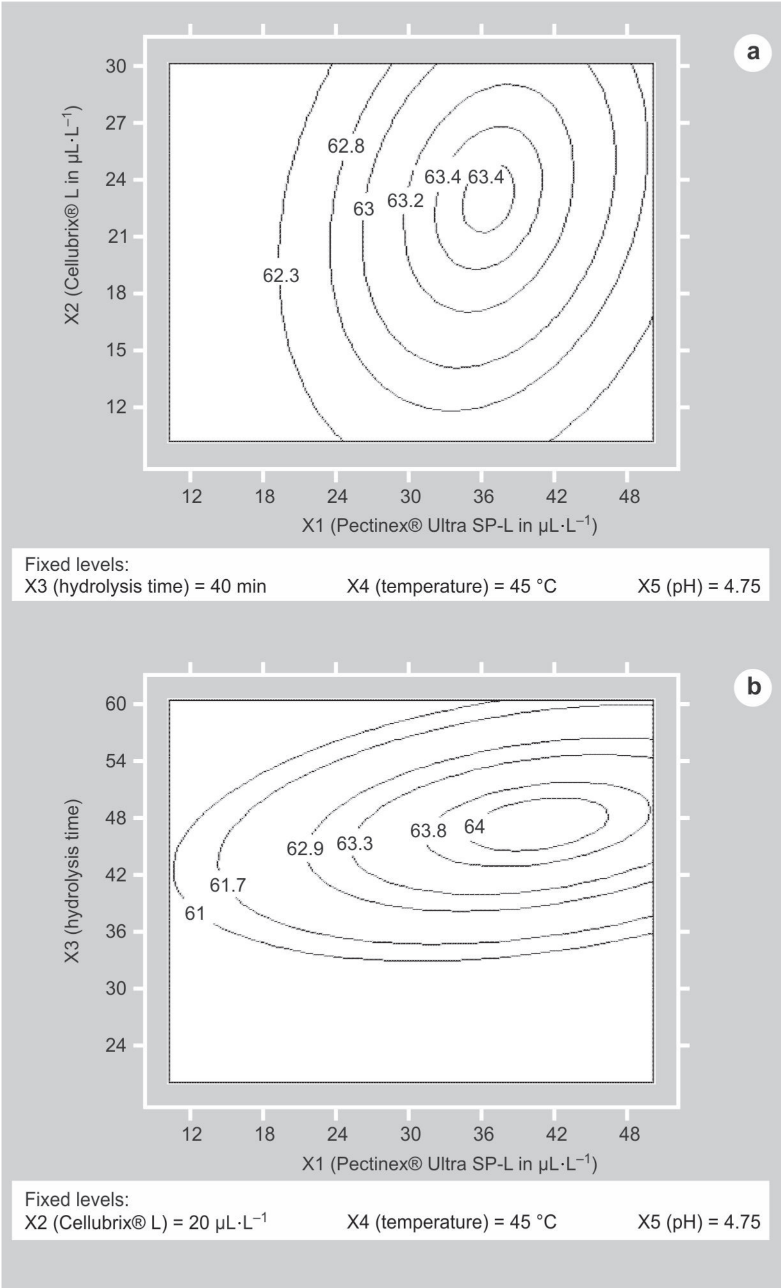


Figure 6.
Superimposed contour plots for optimization of juice yield, cloud stability and the browning index from a combination of:
(a) Pectinex concentration and Cellubrix concentration,
(b) Pectinex concentration and hydrolysis time.

40–46 °C was achieved (*figure 5b*). Based on these results, it can be assumed that the linear positive effect of Cellubrix on the BI is responsible for change in color. Research on different pasteurization methods is still needed for heat stability, as recommended by Cissé *et al.* for baobab fruit nectar [23].

Based on the results of testing (not reported) using different Cellubrix concentrations with a fixed Pectinex concentration (25 $\mu L \cdot L^{-1}$), the BI gradually increased (the color of the cloudy juice became more vivid) with concentrations of Cellubrix, achieving an optimum concentration of 20 $\mu L \cdot L^{-1}$. After reaching the optimal concentration, there was an increase in the cleavage of cellulose and hemicellulose, which induced a decrease in color, affecting the BI. As the BI is positively correlated with yellowness (*b*) and lightness (*L*) [24], the increase in Cellubrix concentration increased the soluble particles and led to the reduction of size in the particles which are mainly responsible for color, consequently decreasing the BI. Based on the results of Sin *et al.*, increasing enzyme concentration and incubation time increase the *L* value [12], but the BI is also affected by *a* and *b* values. This was verified by the positive effects of Cellubrix concentration and hydrolysis time on the BI (*figure 5a*).

3.4. Optimization

During enzyme treatment, maximum juice yield, the highest possible stability and the highest possible BI are desired. Therefore, the optimum cloudy condition was determined by superimposing the contour plots of these three responses upon each other (the superimposed function was built from proportions of Eigenvalues). The contours were generated by selecting two variables, keeping the other variables at their optimum value. The criteria applied for graphical optimization were as follows: (a) maximum juice yield, (b) maximum stability, and (c) maximum browning index. The computer generated plots for juice yield (*figure 2*), cloud stability (*figure 3*) and the BI (*figure 4, 5*), and, using the criteria outlined above, the superimposed plot produced an optimum zone of values (*figure 6, 7*).

For optimization of juice yield, cloud stability and the BI, the superimposed contour plot indicates Pectinex and Cellubrix dosages in the optimized zone to be in the ranges of 28–32 $\mu L \cdot L^{-1}$, and 22–24 $\mu L \cdot L^{-1}$, respectively, when temperature, hydrolysis time and pH were fixed at their central points (*figure 6a*); Pectinex dosage and hydrolysis time in the ranges of 28–32 $\mu L \cdot L^{-1}$

and 42–46 min, respectively, when Cellubrix, temperature and pH were fixed at their central points (*figures 6b*); Pectinex dosage and temperature in the ranges of 28–32 μL·L^{-1} and 45–48 °C, respectively, when Cellubrix, hydrolysis time and pH were fixed at their central points (*figure 7a*); and Pectinex dosage and pH in the ranges of 28–32 μL·L^{-1} and 5.4–5.8, respectively, when Cellubrix, hydrolysis time and temperature were fixed at their central points (*figure 7b*).

Therefore, by overlaying all the responses, the optimum combined set of conditions was found to be 30 μL·L^{-1} of Pectinex concentration and 22 μL·L^{-1} of Cellubrix concentration at 45 °C for 43 min using pH 5.5 (*figures 6, 7*). The responses calculated from the final polynomial functions were y_1 = 68.09 for juice yield, y_2 = 68.35 for cloud stability and y_3 = 29.21 for the BI, respectively, all using comprehensive evaluation and coefficients based on the regression equation.

4. Conclusion

By using response surface methodology and contour plots, an optimum set of operating variables was obtained graphically in order to achieve desired pretreatment levels for cloudy mango juice. Therefore, it is recommended that enzymatic treatment of the cloudiness condition be 30 μL·L^{-1} of Pectinex concentration and 22 μL·L^{-1} of Cellubrix concentration at 45 °C for 43 min using pH 5.5. As we know that mangoes contain cellulase and pectinase, the measurement of these activities will be very useful in the future for more consideration of the added enzymes' activity. Research on correlation between the browning index and the polyphenoloxidase (as well as peroxidase) present in mangoes is needed to find out the kind and ripeness degree of the mango varieties used.

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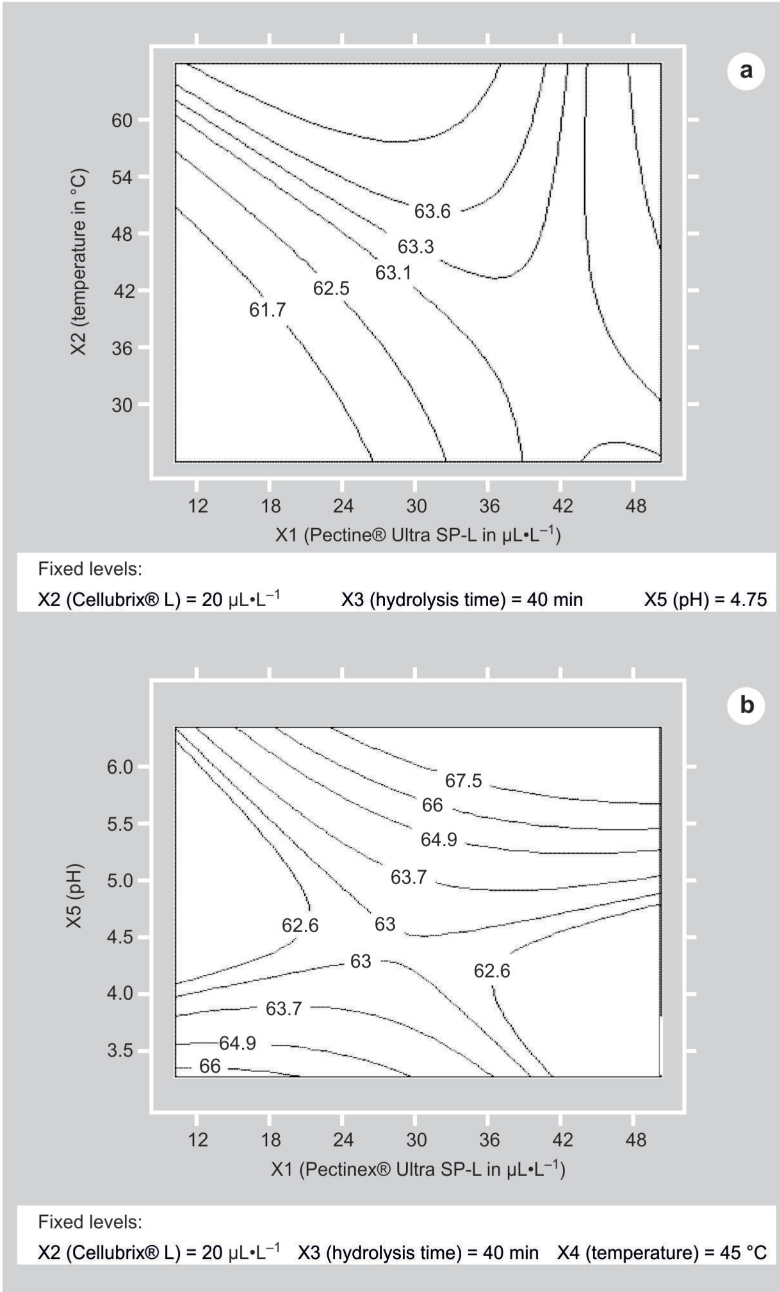

Figure 7.
Superimposed contour plots for optimization of juice yield, cloud stability and the browning index from a combination of:
(a) Pectinex concentration and temperature, (b) Pectinex concentration and pH.

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Optimización de los parámetros para el tratamiento de zumo de mango (*Mangifera indica* L.) naturalmente oscuro, mediante el empleo de enzimas pectolíticas y celulolíticas.

Resumen — **Introducción**. Numerosas características interesantes permitieron producir más zumos de frutas oscuros, de acuerdo con la aceptabilidad del consumidor. La pulpa de mango (*Mangifera indica* L.), una buena fuente de hidratos de carbono y de agua, tiene numerosos usos, entre los cuales, la producción de zumo. A día de hoy, por varias razones, el uso de enzimas agroalimentarias se ha expandido en las industrias del zumo. No obstante, la aplicación de enzimas aún no se ha probado en el contexto de la producción del zumo de mango oscuro, ni siquiera para la estabilización de los néctares. Por ello, el objetivo de nuestro estudio fue el análisis de ciertos parámetros implicados en la producción enzimática del zumo de mango oscuro. **Material y métodos**. Para llegar a nuestro objetivo, empleamos la metodología de superficie de respuestas, gracias a la combinación de cinco parámetros: el tiempo (30–50 min), la temperatura (35–55 °C), el pH (4–5,5), y las encimas, tales como Pectinex® Ultra SP-L (20–40 $\mu L \cdot L^{-1}$) y Cellubrix® L (15–25 $\mu L \cdot L^{-1}$). **Resultados y discusión**. El estudio del rendimiento de zumo, de la estabilidad de la turbidez y del índice de coloración morena mostró que los coeficientes de las correlaciones (valores de R^2) entre dichos caracteres y los parámetros estudiados, eran todos superiores a 0,900. La optimización del rendimiento de zumo, de la estabilidad de la turbidez y del índice de la coloración morena varió en función de los valores específicos, elegidos para los cinco parámetros citados anteriormente. **Conclusión**. Finalmente, se fijó como condición óptima para el tratamiento del zumo de mango oscuro el uso de 30 $\mu L \cdot L^{-1}$ de Pectinex® Ultra SP-L, de 22 $\mu L \cdot L^{-1}$ de Cellubrix® L, a 45 °C, durante 43 min y con un pH de 5,5.

China / *Mangifera indica* / jugo de frutas / procesamiento / propiedades coloidales / estabilizadores / glicosidasas / métodos estadísticos