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Original

Effect of pectinolitic enzymes on the physical properties of caja-manga (*Spondias cytherea* Sonn.) pulp

Efeito de enzimas pectinolíticas nas propriedades físicas da polpa de cajá-manga (Spondias cytherea Sonn.)

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Abstract

Cajá-manga, also known as golden apple and hog-plum, is an exotic fruit native from Îles de la Société (French Polynesia), which was first introduced in Brazil in 1985. The pulp of ripe fruit was treated with the commercial enzymatic pool and its effect was evaluated in terms of yield, as well as the physical properties viscosity, turbidity and color (L^* values). Response surface methodology was used and three levels were adopted for the independent variables temperature (30, 40, and 50 °C), incubation time (30, 60 and 90 minutes) and enzyme concentration (0.01, 0.05, 0.09 v/v%). A central composite statistical design was used to guide the experimental work. The enzyme treatment highly increased both juice yield (up to 56%) and color (up to 8.6%) and strongly decreased viscosity (up to 57.4%), clarity (up to 77%) and turbidity (up to 85.5%). Incubation time was the most interacting facto, whereas temperature was the least one. Optimization analysis was carried out to reduce enzyme concentration to a minimum by superposing the contour plots of the tested properties, and the recommended ranges of the variables enzyme concentration, process temperature and incubation time were, respectively, 0.042-0.068%, 47.0-49.0 °C and 82-90 minutes.
Keywords: *pectinolytic enzymes; Pectinex Ultra SP; cajá-manga pulp; enzyme treatment; juice extraction; juice viscosity.*

Resumo

Cajá-manga, também conhecida como maçã dourada, é uma fruta exótica nativa de Îles de la Société (French Polynesia), a qual foi introduzida no Brasil em 1985. A polpa de frutas maduras foi tratada com enzima comercial e o efeito foi avaliado no rendimento de suco, assim como nas propriedades físicas viscosidade, turbidez e cor (L^*). Metodologia de superfície de resposta foi utilizada e três níveis foram adotados para variáveis independentes: temperatura (30, 40 e 50 °C), tempo de incubação (30, 60 e 90 minutos) e concentração de enzima (0,01, 0,05, 0,09 v/v%). Delineamento estatístico de composto central foi utilizado para orientar o trabalho experimental. O tratamento enzimático aumentou o rendimento do suco (acima de 56%) e a cor (acima de 8,6%), e diminuiu significativamente a viscosidade (acima de 57,4%), clarificação (acima de 77%) e turbidez (acima de 85,5%). O tempo de incubação foi o fator de maior interação, enquanto que a temperatura foi o de menor. Análises de otimização para reduzir a concentração enzimática foram realizadas superpondo as análises de contorno das propriedades testadas, com as variáveis concentração de enzima, temperatura e tempo de incubação que foram respectivamente, 0,042%-0,068%; 47,0-49,0 °C; e 82-90 minutos.
Palavras-chave: *enzimas pectinolíticas; Pectinex Ultra SP; polpa de cajá-manga; tratamento enzimático; extração de suco; viscosidade de suco.*

1 Introduction

Brazil is the third largest world producer of fresh fruit, harvesting about 42 million tons per year (FOOD..., 2009). Nonetheless, the country exports only 2% of its whole production. In addition to the fruits that are usually commercialized, e.g. citrus, apple, banana, and mango, Brazil produces nearly 320 varieties of exotic fruits, although only four varieties are extensively cultivated. The world market for exotic fruits is expanding and presently accounts for 5% of the whole market of fresh and processed fruits. The volume of imported exotic fruits by the USA increased 27% from 1999 to 2006 (UNITED..., 2009). Therefore, processing exotic fruits into pulp and juice is an attractive opportunity for tropical countries to expand their influence over the international market of fruits.

Cajá-manga (*Spondias cytherea* Sonn., Anacardiaceae), also known as golden apple and hog-plum, is an exotic fruit native from Îles de la Société (French Polynesia). It was introduced in Brazil in 1985 and cultivated mainly in the northeast region of the country (DONADIO; MORO; SERVIDOTE, 2000; DONATIO; NACHTIGAL; SACRAMENTO, 1998). The fruit is a prolate spheroid, up to 10 cm in length, in its largest dimension, 9 cm diameter, and 100 g weight. Its skin is golden yellow or brownish, and when ripe its pulp is bitter-sweet and acid. Harvesting time is short and the fruit is consumed either fresh or processed as jelly and juice. Rodriguez-Amaya and Kimura (1989) studied cajá (*Spondias lutea* L.), a fruit quite similar to the cajá-manga, and noticed that the total carotenoids content is higher than the other

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tropical fruits such as cashew, guava, and some varieties of papaya. According to Rosso, Silva and Mercadante (2008) all-trans-lutein was identified as the major carotenoid in cajá-manga, along with 9-*cis*-neoxanthin, all-*trans*-violaxantin, all-*trans*-β-cripto-xantin, 5,6-epóxi-β-cripto-xantin and all-*trans*-β-carotene. The cajá-manga pulp is composed by 72.6-78% moisture, 0.35-0.53% fat, 0.25-1.2% protein, 0.7% ash, 17.8% carbohydrate, 1.5% fiber, 1.2% pectin, 9.3% reducing sugars and 5.0-13.1% soluble solids (°Brix) (LAGO-VANZELA et al., 2011; DONADIO; MORO; SERVIDOTE, 2000).

A serious problem in the industrial juice fruit processing is the release of pectin into the juice. Pectin is a heterogeneous structural polysaccharide present in the cell walls of mesocarp and accounts for up to 4% of the total weight of fresh fruits and up to 35% of cell walls. This complex carbohydrate composed of pectic acid molecules is known for being responsible for giving undesirable characteristics to juices such as high viscosity, haziness, and cloudiness (KASHYAP et al., 2001). The addition of pectinolytic enzymes is the technological route to overcome these undesirable effects besides improving carotenoid extraction from fruit tissues and contributing to cloud stabilization (ABDULLAH et al., 2007; ASKAR et al., 1990; BRASIL; MAIA; FIGUEIREDO, 1995; LEE et al., 2006; LIAO et al., 2007; SANTIN et al., 2008; SHARMA; SARKAR; SHARMA, 2005; SIN et al., 2006).

According to Jayani, Saxena and Gupta (2005) juice extraction and clarification are the main uses for this class of enzyme. Nevertheless, few articles are available about tropical fruit processing using pecntinolytic enzymes; most of them are on enzymatic liquefaction of carambola, banana, mango, and sapodilla (ABDULLAH et al., 2007; CHAUHAN; TYAGI; SINGH, 2001; OLLÉ et al., 1997; RASTOGI; RASHMI, 1999; SANTIN et al., 2008; SIN et al., 2006; SREEKANTAH; JAlEEL; RAMACHANDRA RAO, 1971; SREENATH; NANJUNDASWAMY; SREEKANTIAH, 1987; SREENATH; SUDARSHANAKRISHNA; SANTHANAM, 1995).

The enzymatic hydrolysis of pectic substances is influenced by several factors such as temperature, pH, enzyme concentration, and incubation time (LEE et al., 2006; SIN et al., 2006). Assessing the individual influence of each independent variable is a laborious task, and the Response Surface Methodology (RSM) is a skilful statistical tool for optimizing the search for the best operational condition. This technique was successfully applied to optimize the clarification of carambola, sapodilla, carrot, peach, banana, and mango juices (ABDULLAH et al., 2007; LEE et al., 2006; SANTIN et al., 2008; SHARMA; SARKAR; SHARMA, 2005; SIN et al., 2006; SREENATH; SUDARSHANAKRISHNA; SANTHANAM, 1995; SUN et al., 2006), the extraction of high-ester pectin from passion fruit peel with citric acid (PINHEIRO et al., 2008), the extraction of anthocyanins from black currants (LANDBO; MEYER, 2004), and also to increase juice yield and to decrease turbidity of carrot juices and elderberry (LANDBO; KAACK; MEYER, 2007; SHARMA; SARKAR; SHARMA, 2005), and to reduce mango pulp viscosity (CHAMCHONG; NOOMHORM, 1991), among other applications.

The objective of this work was the application of a commercial pecntinolytic enzymatic pool in the cajá-manga puree in order to evaluate the influence of the variables incubation time, temperature, and enzyme concentration, on the process variables turbidity, clarity and viscosity using RSM to optimize the search in order to help juice processing industries in the production of cajá-manga pulp and juice.

2 Materials and methods

2.1 Materials

Fruit

Fresh cajá-manga was purchased from a local retailer in São José do Rio Preto, SP – Brazil and mature fruits were chosen for this study.

Enzyme

The Pectinex Ultra SP-L (Novozymes - Switzerland AG), produced by *Aspergillus aculeatus*, was applied for the enzymatic treatment. According to the supplier (NOVOZYMES, 2001), the solution enzyme contained 26000 PG U per mL and a range of hemicellulolytic activities, and the optimum activity condition was 3.5 to 6.0 and temperatures below 50 °C. The enzyme was stored at 4 °C before use.

2.2 Methods

For each experiment performed with pulp treated with enzymes, a control sample was prepared with water instead of enzyme solution.

Pulp extraction

The fruits were peeled, deseeded, and blended using a household food blender until a homogeneous fruit pulp was obtained, usually after 3 minutes of blending time. The pulp was then filtered using a domestic plastic sieve. The total dissolved solid concentration of the obtained pulp was 13.1°Brix, measured with a hand held refractometer (Pocket Refractometer PAL-3). The pulp was kept frozen in plastic bags at -20 °C before use. For the experiments, the bags were removed from the freezer and left to thaw at room temperature.

Enzyme treatment

The samples, corresponding to 30 g of pulp each, were submitted to enzymatic treatments under different conditions. The adopted ranges for controlled variables were based on preliminary assays; three levels were adopted for the variables: incubation time, temperature, and enzyme concentration. A statistical experimental design, further described, was used to guide the experimental work. The experiments were conducted in Erlenmeyer flasks which were partially immersed in water and kept at constant temperature in a thermostatic bath. The enzymatic treatment was halted inactivating the enzyme by heating the suspension at 100 °C for 5 minutes.

After the treatment, the pulps were then centrifuged at 6000 g for 10 minutes (Avanti J-25, Beckman Coulter, USA) and the supernatant was collected for further analyses. The pH of cajá-manga was considered constant along the experiments at its natural value (3.3), which was measured using a calomel electrode pHmeter.

Physical and chemical analysis

Turbidity

Turbidity was assessed using a Turbidimeter (Model DR/2000, Hach Company), and the results were reported as Nephelometric Turbidity Units (NTU).

Clarity

Clarity was determined by measuring absorbance at 660 nm using a UV–Vis spectrophotometer (Beckman DU. 640 Spectrophotometer - USA); water was used as reference.

Viscosity

Apparent viscosity was determined using a Brookfield viscometer (DV-III+ Rheometer, USA) with a 40 cone and plate spindle. The measurements were made at a constant angular velocity (130 RPM). These experiments were conducted at constant temperature (10 ± 1 °C) surrounding the spindle with a water jacket.

Color measurements

Sample colors were estimated by taking pictures of the samples with a digital camera (Mitsuca DC 7325BR, 3.0 megapixels), and the digitalized pictures were analyzed using the software LensEye V-01 (Engineering and Cyber-Solutions, Gainesville, FL-USA). The adopted property was luminosity (L^*), according to the CIELAB color scale. To avoid external interference on the sample colors, the pictures were taken in a chamber assembled according to the suggested design of Luzuriaga, Baiaban and Yeraian (1997). The samples were placed in opaque white pots (4 cm diameter × 3 cm height), close to the color reference cards (*X-Rite Munsell-Color, Color Checker)* to be compared with the reference sample using a software; and next, the parameter L* was determined.

Yield

Yield was defined as the extracted juice volume. The enzymatic treated pulp was centrifugated at 6000 g (Avanti J-25, Beckman Coulter, USA), and the volume of the supernatant obtained was measured using a digital caliper.

2.3 Statistical experimental design

The experimental work followed a central composite statistical experimental design (COCHRAN; COX, 1957; MYERS; MONTGOMERY, 2002), and the variance analysis (ANOVA) was performed using the software Statistica 7.0 (Statsoft, Tulsa, OK - USA). As previously mentioned, three independent variables were employed (enzyme concentration, incubation time, and temperature) at three levels and five replications were done at the central point, resulting in 19 experimental runs. The uncoded values of those variables are their actual values, indentified by the capital letter X, whereas the coded values are represented by the levels -1, 0 and 1, identified by the lowercase letter x. Table 1 shows the correspondence among the uncoded and coded variables. A full quadratic model, including cross-product terms, was fitted to the experimental data, which can be represented by the following Equation 1:

$$Y = a_0 + \sum_i a_i x_i + \sum_i a_{ii} x_i^2 + \sum_i \sum_j a_{ij} x_i x_j + \varepsilon \quad (1)$$

where Y is the response variable; $a_{0,}$ a_i, a_{ii}, and a_{ij} are the regression coefficients, and ε is the error, also quoted as the model's non-explained portion.

Table 2 shows the design matrix. The experiments were randomly executed. Surface response figures were drawn by keeping one variable fixed at the central point and varying the other two within the whole range.

3 Results and discussion

Table 3 shows the experimental results for the measured response variables, and the referred runs can be seen in Table 2.

Table 1. Correspondence between coded and uncoded variables.

Independent uncoded variables (X)	Independent coded variables (X)		
	-1	0	1
Incubation time (minute) – X_1	30	60	90
Incubation temperature (°C) – X_2	20	40	60
Enzyme concentration (%) – X_3	0.01	0.05	0.09

Table 2. Statistical experimental design matrix.

Run	Incubation time (minute)	Temperature (°C)	Enzyme concentration (%)
	X_1	X_2	X_3
1	30	40	0.05
2	90	40	0.05
3	60	30	0.05
4	60	50	0.05
5	60	40	0.01
6	60	40	0.09
7	90	50	0.09
8	30	50	0.09
9	90	30	0.09
10	30	30	0.09
11	90	50	0.01
12	30	50	0.01
13	90	30	0.01
14	30	30	0.01
15	60	40	0.05
16	60	40	0.05
17	60	40	0.05
18	60	40	0.05
19	60	40	0.05

The regression estimated parameters and the correlation coefficient (R^2) are shown in Table 4, where one may notice that the R^2 values are satisfactory considering that three independent variables were used for the fitting process. It is worth mentioning that R^2 was calculated considering all terms of the full quadratic model significant, and that if only the significant terms at a specific significance level were chosen for a subsequent fit, the values of R^2 would certainly be poorer. In Table 3, AVG is the average value of the 19 tests of the control samples, SD is the standard deviation for the average, and CI is the confidence interval at 95% significance level assuming normal distribution of the experimental values.

Noticeable results must be emphasized. Considering maximum variations when comparing the treated material to the control samples, turbidity decreased up to 85.5% and viscosity

Table 3. Experimental results of the enzymatic treatment.

Run		Response variable				
		Turbidity (NTU)	Clarity (abs)	Viscosity (Pa s)	Yield (mL)	Color (L^*)
1		128.5	0.103	4.98	16.4	97.97
2		100.0	0.093	2.92	15.4	99.55
3		135.5	0.122	4.45	16.0	98.02
4		155.5	0.112	4.31	15.9	98.68
5		152.5	0.149	4.44	15.3	97.47
6		142.0	0.111	4.43	16.5	97.31
7		102.5	0.096	6.15	15.3	98.74
8		143.5	0.122	5.64	16.0	97.61
9		106.0	0.108	4.78	15.3	99.74
10		136.0	0.122	3.93	16.0	97.74
11		109.5	0.113	7.15	14.5	98.95
12		187.5	0.168	8.20	14.5	99.02
13		136.0	0.126	4.29	14.3	99.01
14		213.0	0.232	6.07	14.0	98.21
15		115.0	0.102	4.16	16.4	98.18
16		129.5	0.117	3.49	15.8	97.91
17		134.0	0.127	3.74	16.3	97.63
18		116.5	0.115	4.55	16.8	98.17
19		128.5	0.116	4.10	16.3	98.55
Control	AVG	1467.9	1.006	19.25	10.7	91.8
	SD	342.2	0.208	3.70	1.4	1.29
	CI	153.8	0.094	1.66	0.6	0.018
Maximum Variation (%)		85.5	77.0	57.4	56.8	8.6

Table 4. Estimated parameters of the regression analysis.

Parameter	Related variables	Turbidity (NTU)	Clarity (abs)	Viscosity (Pas)	Yield (NTU)	Color (L* value)
a_0	-	127.3144***	0.111827***	3.836134***	16.30284***	98.03526*
a_1	X_1	-25.4500***	-0.021085**	-0.352500	-0.22500*	0.54400***
a_{11}	X_1X_1	-16.3325	-0.009335	0.322448	-0.46263*	0.78567**
a_2	X_2	-2.8000	-0.009765	0.792500**	0.06250	0.02800
a_{22}	X_2X_2	14.9175	0.009515	0.752448	-0.40013*	0.37567
a_3	X_3	-16.8500**	-0.022775***	-0.522000	0.65000***	-0.15250
a_{33}	X_3X_3	16.6675	0.022765*	0.809948	-0.46263 *	-0.58683*
a_{12}	X_1X_2	-1.5000	0.004656	0.047500	-0.03125	-0.21687
a_{13}	X_1X_3	10.5000*	0.015181*	0.525000	-0.21875	0.30063*
a_{23}	X_2X_3	7.0000	0.008181	-0.238750	-0.09375	-0.23437
R^2	-	0.90	0.90	0.84	0.94	0.90
Optimum	X_1	43.50	43.88	75.78	46.99	49.82
	X_2	39.0	43.82	34.68	40.01	38.08
	X_3	0.078	0.074	0.053	0.082	0.043
	Y	128.5	0.109	3.52	16.61	97.96

Level of significance: *5%, **1%, ***0.1%.

up to 57.4%, whereas all the following variables increased: clarity up to 77%, lightness up to 8.6%, and yield up to 56%. This is an indicative of the high amounts of pectic substances in the cajá-manga pulp, and it also indicates the efficiency of the chosen pool of enzymes for this application.

Incubation time was the variable which significantly affected the largest number of response variables, followed by enzyme concentration. Except for viscosity, temperature was not a very interacting factor since temperature stability is expected for commercial enzymes.

3.1 Turbidity

Only linear terms of incubation time, enzyme concentration and cross product, of these variables were significant, as shown in Table 4. Cajá-manga juice turbidity was quite higher than the other exotic fruit juices such as carambola (ABDULLAH et al., 2007) and sapodilla (SIN et al., 2007). Turbidity directly indicates unsettle matter or impurities in water suspension, such as colloidal polysaccharide particles in fresh juices (GRASSIN; FAUQUEMBERGUE, 1999). For orange and tomato juices, this property is a positive sensorial aspect, whereas for pear, guava, apple, and carambola juices it is a negative one (ABDULLAH et al., 2007; CHOPDA; BARRETT, 2001; ISABELA; GERALDO; RAIMUNDO, 1995; KASHYAP et al., 2001; MIHALEV et al., 2004; SIN et al., 2007). During a non rigorous sensorial analysis carried out in the lab, the testers stated that clear cajá-manga juices are more attractive than the cloudy ones.

Figure 1 shows fitted surface to the experimental data and the contour plot for Turbidity. From the contour plot, one might realize that the predictable variables are correlated since a non symmetrical pattern is observed. Figure 2 presents the normal plot, in which randomly distributed residuals can be noticed, a common behavior of all other residuals obtained in the present study. Both independent variables incubation time and enzyme concentration negatively affected turbidity, making the juice clearer, although the cross product presented a positive effect. In fact, long exposition to high enzyme concentrations are likely to break down pectic substances exposing positive nucleus sites to surrounding negative charges, settling out the so formed large protein-pectin particles (KASHYAP et al., 2001). A closer view of the turbidity dependence on incubation time reveals that, at low enzyme concentration, turbidity continually decreases as time increases but, at high value of enzyme concentration, turbidity increases for short times reaching a maximum at about 40 minutes, and thereafter it decreases. Sin et al. (2006) also observed similar effects for sapodilla juice and explained this behavior as the formation of a protein-carbohydrate complex or unsettling particles (protein-tannin).

Some authors reported similar synergistic contribution of incubation time and enzyme concentration to turbidity. Abdullah et al. (2007), Liao et al. (2007) and Landbo, Kaack and Meyer (2007) observed reductions in juice turbidity for carambola (48% reduction), carrot (22%), and elderberry (30%), respectively, using commercial enzymatic pools.

3.2 Clarity

Clarity was influenced only by incubation time and enzyme concentration. This result was predictable since the turbidity trend was known since both measurements are based on a similar physical principle, which is the attenuation of an incident radiation. Turbidity indicates the light scattered at an angle of 90 degrees from an incident beam, whereas clarity (also absorbance and transmittance) provides the absorbed radiation from an incident beam, which traveled a straight path. However, when measuring the absorbance of solutions with suspended solids, part of the incident radiation is scattered rather than absorbed masking the measured values. Hence, clarity is a more adequate property than turbidity when clear juices are being processed. Different commercial pectinases have been successfully used to clarify juices such as banana (LEE et al., 2006), sapodilla (SIN et al., 2006), black currant (LANDBO; MEYER, 2004), guava (CHOPDA; BARRETT, 2001), prickly pear (ESSA; SALAMA, 2002), tangerine (CHAMCHONG;

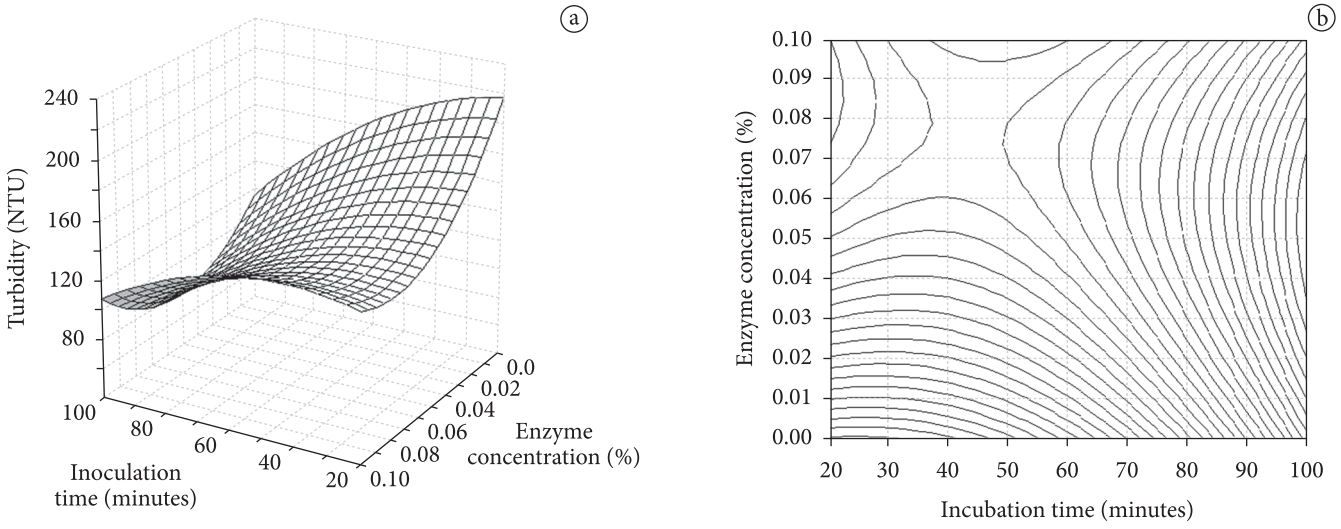

Figure 1. Response surface (a) and contour plot (b) for turbidity.

NOOMHORM, 1991), and apple juice (BEVERIDGE; WEINTRAUB, 1997).

Figure 3 shows the response surface for clarity, where it can be seen that the surface shape is quite similar to the turbidity one. Sin et al. (2006) and Rastogi and Rashmi (1999) observed a similar dependence for the significant independent variables incubation time and enzyme concentration when studying the liquefaction of sapodilla and mango pulp using RSM at constant temperature (42 °C). The significant terms and trends observed by Sin et al. (2006) for clarity exactly matched the ones here noticed, whereas Rastogi and Rashmi (1999) did not mention the quadratic incubation time term as significant.

3.3 Yield

Yield was influenced by all main effects of all tested variables although only enzyme concentration presented a positive effect over juice extraction. No cross-product terms were significant. Figure 4a-c shows the surface response for juice yield, where one may notice that all curves present similar quadratic shapes.

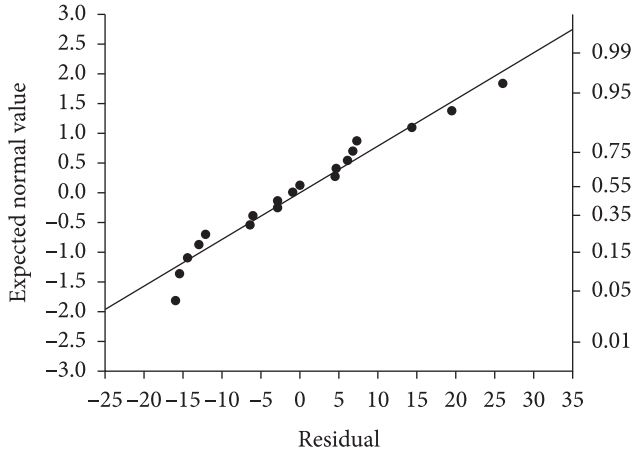

Figure 2. Normal plot for turbidity.

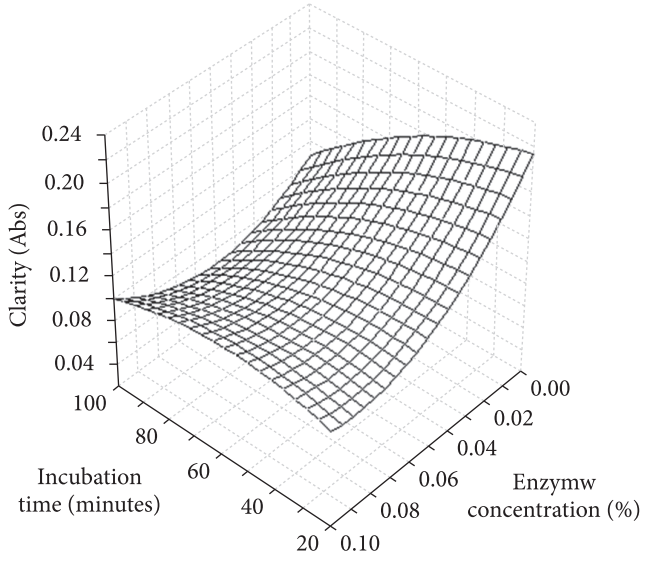

Figure 3. Surface response for clarity.

Rastogi and Rashmi (1999) observed a similar effect for incubation time and enzyme concentration when liquefying mango pulp. They worked at constant temperature and did not mention the quadratic enzyme concentration term. Sun et al. (2004) enzimatically treated carrot juice at constant room temperature and also observed an intense relationship between the independent variables incubation time (X_1) and enzyme concentration (X_3). They also obtained the same response surface shape as the one found in this work and realized that the linear term of both X_1 and X_3 presented positive effect over the juice yield, whereas the quadratic term of X_3 presented a negative effect. Sharma, Sarkar and Sharma (2005) also treated carrot juice and noticed that the main effects of the variables incubation time, incubation temperature, enzyme concentration, and cellulase/pectinase concentration ratio positively affected juice yield, whereas the significant interactive effects negatively affected juice extraction. From these conflicting results, one may conclude that, although RSM allows for the statistical identification of treatments that affect juice yield, understanding how the adopted independent variables and their cross interactions interfere in the extraction is not a simple task since different fruits present different chemical links among their constituents, as well as distinct enzymatic pools act distinctly on the constituents, even for the same fruit. Therefore, specific experiments conducted by RSM are mandatory to assess the intrinsic relationship between specific enzymes and fruits; and biochemical studies are necessary to understand the fruit structure and the enzyme action.

It was expected that both incubation time and temperature would present a positive effect on juice yield; however, only enzyme concentration was positive. One might speculate that enzyme exposure to high temperatures for a long period might lead to a deleterious effect on enzyme stability considering that the enzyme was produced from a mesophilic mold. Hence, further studies must be carried out under moderate and high temperatures to confirm this hypothesis. The positive effect of enzyme concentration is understandable since a synergistic action of the enzymes present in Pectinex Ultra SP-L enzymatic pool is expected, which would act on other compounds such as cellulose, hemicellulose, starch, protopectin, and pectin releasing the juice.

Sun et al. (2006) achieved an increase of 48.1% in carrot juice yield using Pectinex Ultra SP-L, and obtained an even higher yield (71,5%) using the enzymatic pool Cellulase FNC-1 (Beijing Funong Food Co., China). Sharma, Sarkar and Sharma (2005) prepared their own enzymatic pool from *Aspergillus foetidus* (pectinolytic) and *Thrichoderma reesi* (cellulotityc) and increased the carrot juice extraction up to 25%. On the other hand, Liao et al. (2007), using Pectinex Smash XXL (Novozymes), improved carrot juice extraction by 20%. Essa and Salama (2002) cleared prickly pear juice using commercial pectinase and extracted up to 32% more juice when compared to the control samples. Sreenath, Sudarshanakrishna, Santhanam (1994) found that the addition of a cellulase and pectinase recovered up to 86% of pineapple juice, whereas non-enzimatically treated samples recovered 72%. Hence, the maximum improvement on yield, when compared to the control samples obtained in the present study (57%) is within the results published in the literature.

3.4 Viscosity

It is well known that fruit pulps present non-newtonian rheological behavior (URLAUB, 1996). Nevertheless, assessing the cajá-manga juice rheological behavior for all the statistical treatments applied in this study would be time consuming. A good example of this kind of comprehensive rheological research may be found in Bhattacharaya and Rastogi (1998) for mango pulp.

For the apparent viscosity, an odd behavior was observed since only the linear temperature term was significant. It must be emphasized that all viscosity measurements were conducted at the same constant temperature (10 °C) and only the enzyme tests were carried out at variable temperatures. Sreenath, Sudarshanakrishna and Santhanam (1995) noticed that the enzyme concentration strongly influenced the viscosity reduction when Pectinex and Celluclast (both Novozymes) were applied, achieving up to 80% viscosity reduction. Lee et al. (2006) used Pectinex Ultra SP-L for banana juice clarification, and the statistical analysis showed that the main effects (linear and quadratic) of the enzyme concentration, incubation temperature, and incubation time were significant. Brasil, Maia and Figueiredo (1995) reported a remarkable reduction of 62.9% in the guava juice viscosity when Clarex-L super-concentrate (Miles-Brasil, Brazil) was applied. The results obtained by Sharma, Sarkar and Sharma (2005) also indicated that not only temperature, but also enzyme concentration and incubation time affected carrot juice viscosity when Pectinex Smash XXL was employed achieving up to 41% reduction. Some authors (KASHYAP et al., 2001; URLAUB, 1996) claimed that pectic substances present water holding capacity and that enzyme action would break down the pectin chains releasing captive water and thus reducing viscosity.

It should be highlighted that the viscosity of cajá-manga pulp is quite higher than those observed for the pulps of the above mentioned studies. Viscosity is a far more complex

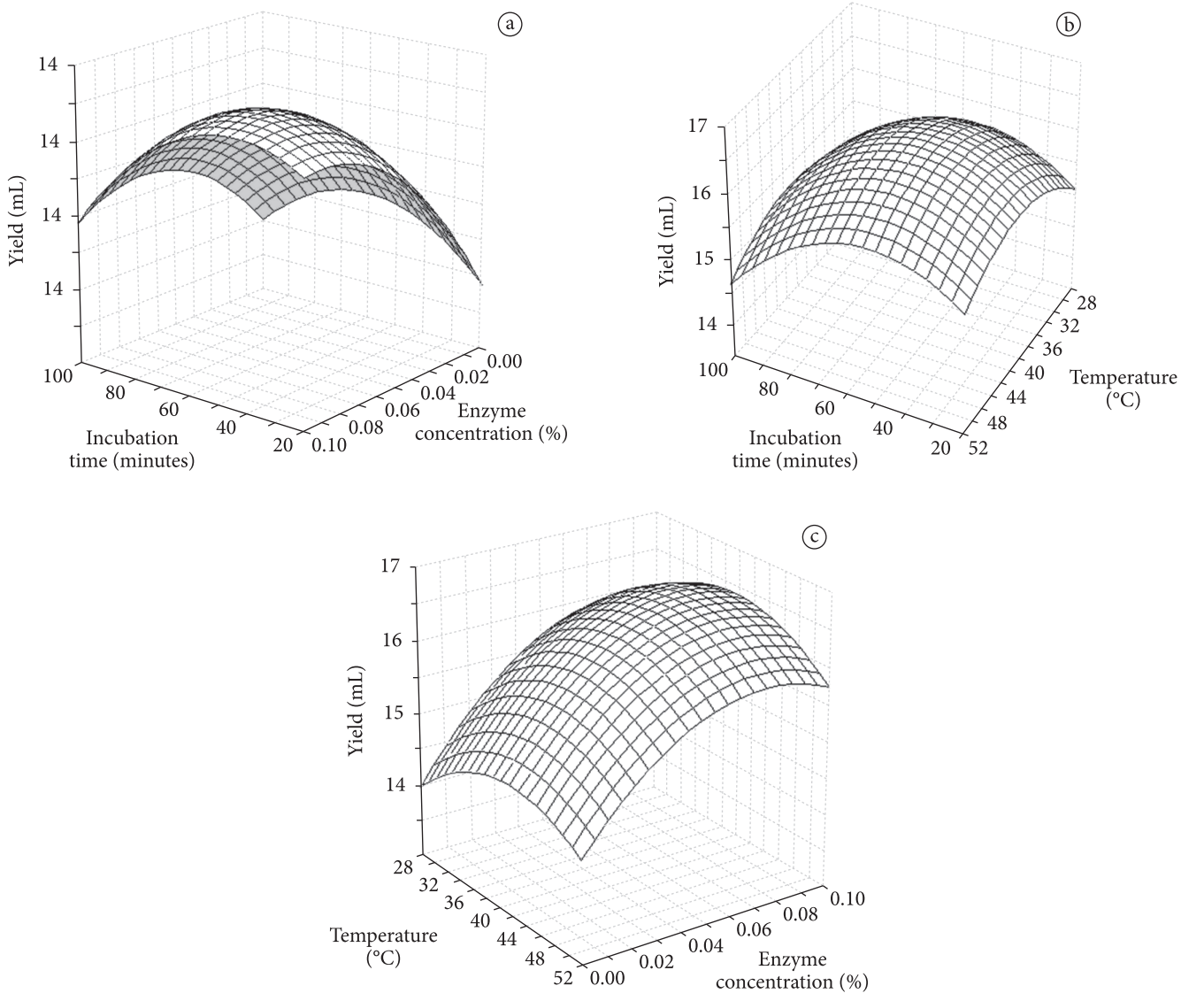

Figure 4. Surface response for cajá-manga juice yield, a) incubation time × enzyme concentration, b) incubation time × temperature, and c) temperature × enzyme concentration.

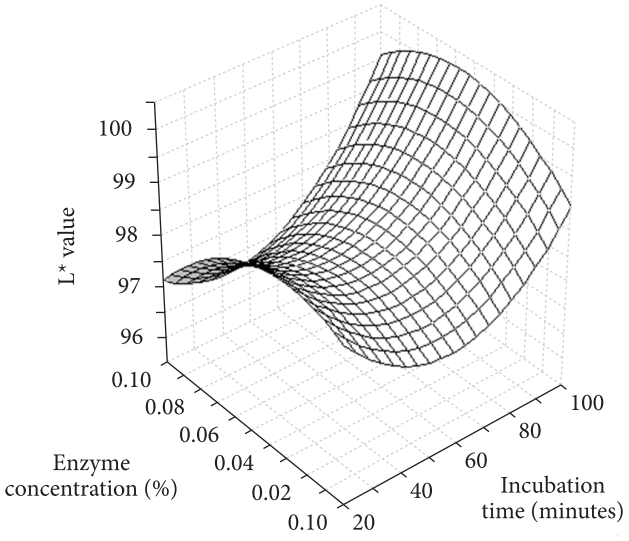

Figure 5. Surface response for lightness.

property than the others tested in this work because it is measured under sample movement and therefore it does not represent only a physicochemical interaction, but a dynamic interaction as well. Ergo, specific studies must be carried out with higher enzyme concentrations and incubation times to verify possible undisclosed effects.

3.5 Color (L)*

Lightness presented almost the same dependence as Turbidity and Clarity since they are similar properties and somehow measure the interference of the sample in an incident ray. Only incubation time presented a positive effect on lightness, and the maximum improvement observed for L^* values was nearly 9%. Liao et al. (2007) claimed that the enzyme treatment with Pectinex Smash XXL increased carrot juice lightness. Abdullah et al. (2007) reported that carambola juice lightness increased with the enzyme concentration, but it decreased after 80 minutes incubation time. These authors argued that the

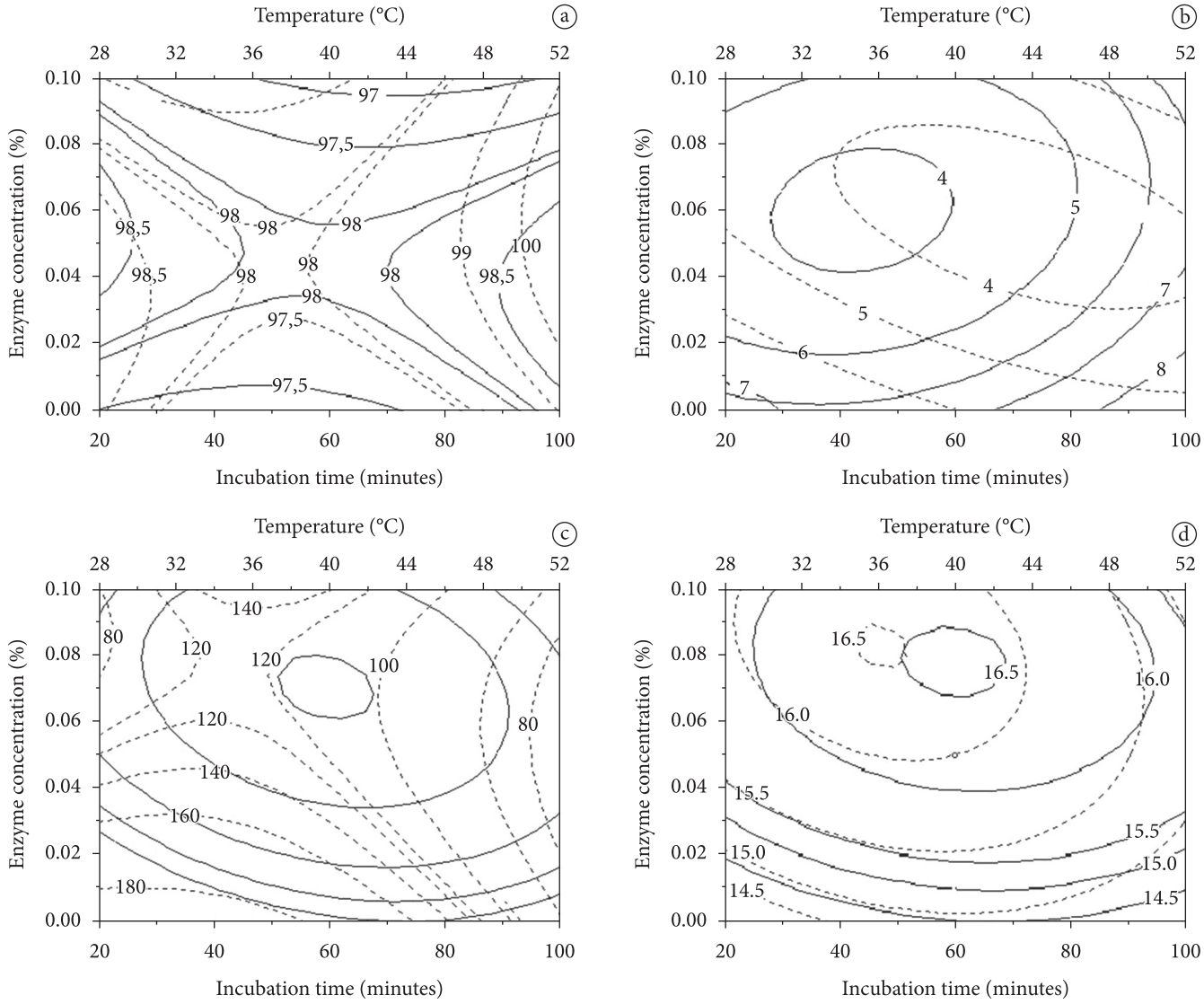

Figure 6. Optimum regions for the contour plots (a- L^* value; b- viscosity; c- turbidity; d- yield, ---- time, —temperature).

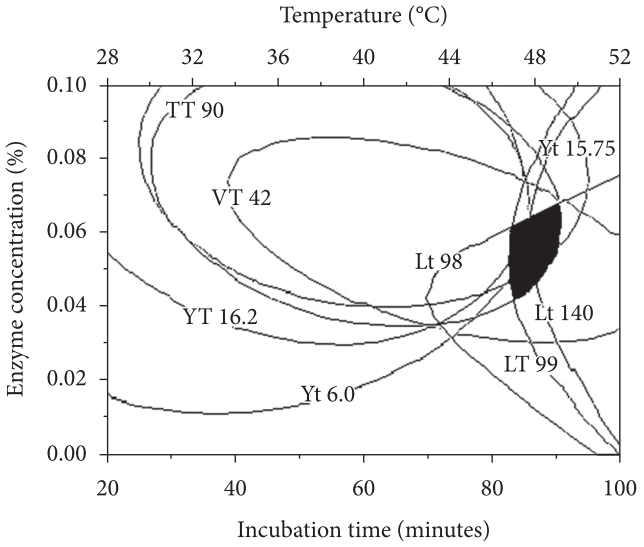

Figure 7. Superimposed contour plots for optimization of enzyme concentration (T = turbidity, V = viscosity, Y = yield, L = color, t = temperature, T = time).

formation of protein-tannin complex would negatively affect the L^* value although they did not present any evidence of this fact. Oddly, the significant parameters of Abdullah and co-workers statistical model were not the same observed for Turbidity and Clarity. Sin et al. (2006) informed that only the quadratic effects related to incubation time, incubation temperature, and enzyme concentration were significant for sapodilla juice lightness, similarly to Turbidity and Clarity.

Figure 5 shows the surface response of lightness for enzyme concentration and incubation time, where one may note the saddle shape, similar to that obtained by Abdullah et al. (2007).

3.6 Optimization

Even though the purpose of using enzyme in fruit process is to improve the physicochemical characteristics of the product, cost is a crucial industrial factor to be taken into account in order to achieve a reasonable operational condition. Among the independent variables adopted in this study, enzyme concentration is the one which has the greatest impact on the process cost. Thus, lower enzyme concentrations were used as a criterion to optimize the search for the ideal operational conditions. Considering that turbidity and clarity presented quite similar behavior, only turbidity was chosen for this optimization procedure. Figure 6 (a) to 6 (d) shows the optimal condition for each property investigated, where the continuous lines represent the enzyme concentration as a function of temperature, whereas the dashed lines represent the enzyme concentration dependence on incubation time. These figures show the maximum juice extraction and color, and minimum viscosity and turbidity. The optimum region, Figure 7, was obtained by superimposing the contour plots presented in Figure 6. The optimized zone depicts enzyme concentration (%) in the range 0.042-0.068%, process temperature 47.0-49.0 °C, and incubation time of 82-90 minutes. In Figure 7, the first letter represents the tested variable (T for turbidity, V for viscosity, Y for yield, and L for L^* value), and the second the independent variable (T for temperature and t for incubation time). The number is the maximum or minimum value of the tested variable.

4 Conclusions

The commercial enzymatic pool Pectinex Ultra SP-L was efficient in reducing the cajá-manga pulp turbidity and viscosity and in enhancing clarity and lightness besides improving the juice yield. Incubation time was the most interacting factor, followed by enzyme concentration and temperature. The response surface methodology and the graphical method indicated that the application of commercial pectinases to cajá-manga juice extraction should be carried out under enzyme concentration (%) between 0.042-0.068%, temperature between 47.0-49.0 °C and time process between 82-90 minutes.

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