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Effect of Applying Pectinolytic Enzymes in *Spondias tuberosa Arr. Cam.* Pulp

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Efeito da Aplicação de Enzimas Pectinolíticas em Polpa de *Spondias tuberosa Arr. Cam.*

Resumo: Umbu (*Spondias tuberosa Arr. Cam*.) é uma fruta nativa das regiões semiáridas do nordeste brasileiro que vem despertando interesse do setor de alimentos de frutas exóticas devido a sua qualidade nutricional e sensorial, e com isso aumenta-se a variedade de produtos que utilizam umbu como matéria-prima. Com o objetivo de verificar o efeito da aplicação de enzimas pectinolíticas sobre a cor e a viscosidade da polpa de umbu, foi aplicado um planejamento composto central com 3 variáveis independentes (tempo, temperatura e concentração de enzima) e 3 variáveis dependentes (viscosidade, L* e b*) utilizando polpa de umbu em 3 diferentes pH (2,3, 3,5 e 4,5). Os resultados significativos foram com o pH 2,3 para a resposta viscosidade e com o pH 3,5 para o L*. Com o pH natural da fruta (2,3), o tempo e a temperatura foram as variáveis significativas para a viscosidade, e no pH 3,5 a concentração enzimática e o tempo foram as variáveis que influenciaram a luminosidade da polpa, dentro dos valores testados. A aplicação destas enzimas exerceu efeito na redução da viscosidade e no processo de clarificação da polpa de umbu. Deve-se, portanto, considerar a relação de pH, temperatura e concentração enzimática para definição dos parâmetros técnicos de aplicação industrial às polpas de frutas.

Palavras-chave: Umbu; enzimas pectinolíticas; design experimental; valor L*; viscosidade.

Abstract

Umbu (*Spondias tuberosa Arr. Cam*.) is a native fruit from the Brazilian semi-arid regions of north-eastern, which has been raising the attention of the exotic fruit sector due to its nutritional and sensorial qualities, increasing the range of products using umbu as raw material. With the aim to verify the effect of applying pectinolytic enzymes on colour and viscosity of umbu pulp, an central composite design with three independent variables (viscosity, *L** and *b**) and three dependent variables (viscosity, L* and b*) was applied using umbu pulp at three different pH values (2.3, 3.5 and 4.5). The most significant results were found with pH 2.3 for viscosity response, and pH 3.5 for *L**. With the fruit's natural pH of 2.3 time and temperature were the most significant variables for viscosity. With pH 3.5 enzymatic concentration and time were the variables that influenced the pulp's luminosity, within the tested values. The application of these enzymes exerted effect on reducing viscosity and in the process of clarifying the umbu pulp. Therefore, it should be consider the pH ratio, temperature and enzyme concentration to define the technical parameters of industrial application to umbu fruit pulp.

Keywords: Umbu; pectinolitic enzyme; experimental design; L* value; viscosity.

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Effect of Applying Pectinolytic Enzymes in *Spondias tuberosa Arr. Cam.* Pulp

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1. Introduction

The north-eastern region of Brazil is known for large scale production of various tropical and sub-tropical fruits with differentiated sensorial and nutritional characteristics and high potential for economic exploration. As an example, we can highlight the umbu fruit (*Spondias tuberosa* Arr. Cam.), which is widely commercialized, especially in its pulp form and *in natura*.[1-3] In 2010, 9.804 tons of the fruit were collected, with the state of Bahia as the largest producer with 89% of the national production, Pernambuco with 4% and Rio Grande do Norte with 2%.[4] In 2012, 22 tons of umbu were industrialized in the form of pulps, jams, compotes, mousses and sweets.[5]

The products derived from umbu (juices, jams, ice-creams, sweets) have a good acceptance in the national and international markets, due to its bittersweet taste and aromatic quality, considered an exotic flavoured product. The yield of the fruit in pulp is considered high, varying from 62% to 75%, depending on the fruit maturation.[6] Chaves *et al.* [7] found 1,1% pectin in the fruit, which places it in the category of fruits with a high pectin content. The presence of pectin in juices or pulps causes some inconveniences such as blocked filters and pipes, incrustation, and damaged equipment, besides phase separation (sedimentation) of industrialized products. Commercial enzyme preparations, usually contain the main hydrolytic enzymes important to minimize these processes, such as pectinases, celluloses and hemicelluloses.[8] The enzymatic treatment alone, or followed by other variables of the process, plays a fundamental role in the physical-chemical characteristics of the juice, especially on the improvement of yielding, colour, quality and product acceptance.[9,10]

Pectinases are a group of enzymes that contribute to the breakdown of pectic materials.[11] This phenomenon may be linked to the paradox that the pectinolytic enzyme attack can result in two different events:[12] the pectinases catalyze the degradation of high molecular weight pectin structures into small pectin fractions. The resulting small, negatively charged, pectin fractions may be able to stay in suspension and contribute to increase the immediate turbidity, due to an increased scatter effect of small particles as compared to larger.[2] The pectinases act on the pectin layer encapsulating the protein core of proteinaceous pectin particles in suspension. This action results in an electrostatic agglomeration of oppositely charged particles that may lead to transient turbidity increase, and subsequently results in precipitation of the agglomerated complex, resulting in decreased turbidity. Pectinases also allow the release of other substances that influence the quality (including color and aroma) from the cells structures, by maceration.[10,13] The applications of these enzymes in the food have been reported in ripening fruit, clarification and reduction of viscosity in fruit juices, preliminary treatment of grape juice and increased pulp extraction rate.[14-19] The use of pectinolytic enzymes is an alternative for increasing the yield of pulp extraction process, decrease processing time, reduce fouling in pipes and equipment, optimize the filtration process and, in addition, the process of clarification of juices enables the supply a noble product of crystalline appearance.

It should be highlighted that drinks derived from fruits are an excellent source of phytochemical, vitamins and minerals compounds. Besides that, they present a high hydration capacity.[20,21] The objective of this work was to evaluate the effect of applying commercial pectinolytic enzyme on the colour and viscosity of the umbu pulp, using the response surface methodology, having as variables: pulp pH, enzyme concentration, time and temperature of application.

2. Materials and Methods

The umbu fruits were collected in the region of Uauá, Bahia, Brazil, and processed in the Food and Drink Technological Area (Fruit Processing Plant), of the National Service of Industrial Learning (SENAI), in Salvador, Dendezeiros Unity. The fruits were selected, washed, sanitized (100ppm active chloride for 20 minutes), de-pulped (Itametal Compacta de-pulping machine, with 1CV engine) and sieved (1mm sieve), obtaining the pulp separate from the bagasse (skin and seeds). The pulp was filtered through a cheese cloth (Morin fabric) to remove suspended particles (skin and seeds) and stored at - 6°C in a vertical freezer (Eletrolux) until analysis. The batch was divided in three parts, of which two received pH correction to 3.5 and 4.5 (batch 2 and 3 respectively) with citric acid, and the last part maintained the fruit’s natural pH of 2.3 (batch 1).

In order to optimize the application of pectinolytic enzyme on umbu pulp, the effect of concentration of pectinolytic enzyme, time and temperature of the treatment were verified through a rotational central composite design (DCCR 2^3) with three repetitions in the central point, totalling 17 assays. As dependent response variables, viscosity and colour of the pulp treated by the enzymes were evaluated on the three batches obtained. The parameters of optimization of enzymatic application with real and codified values and design matrix can be found on Table 1 (the adopted ranges for controlled variables were based on preliminary assays). The enzyme used in the experiments was Pectofruit (Endozym-France, a commercial enzyme preparation from *Aspergillus niger* (OMG-free) used in the industry for fruit juice processing).

The experiments were conducted in plastic tubes containing 30g of pulp for each assay of the experimental design (Table 1), which were partially immersed in water and kept at constant temperature in a thermostatic bath (Marconi). The enzymatic treatment was halted by inactivating the enzyme through heating of the suspension at 100ºC for 5min. After enzymatic treatment the apparent viscosity was determined using a Brookfield viscometer (DV-I+ Rheometer, USA) with a SL1 spindle and 16mL of umbu pulp sample. The measurements were made at a constant angular velocity (30rpm). These experiments were conducted at constant room temperature. The colour of umbu pulp after enzymatic treatment was measured using Chroma Meter CR 400/410 (Konica Minolta), using scales L* and b*. The Commission Internationale de l'Eclairage, (CIE) system reference measures the lightness (L* value) on a numerical scale, where White = 100 and Black = 0 and +b* is the yellow direction, and -b* is the blue direction. The pulp samples (10mL) were placed in quartz for colour analysis.

The influence of the enzymatic treatment conditions on viscosity and colour of umbu fruit pulp were evaluated by the creation of response surfaces, using the experimental design module and ANOVA (variance analysis) at a 95% confidence level of the statistical software Statistica for Windows version 7.0.

Table 1. Rotational central composite design matrix

Assays	Enzyme concentration [ppm]	Temperature [ºC]	Time [min]
	X_1	X_2	X_3
1	-1 (8)	-1 (35)	-1 (48)
2	+1 (20)	-1 (35)	-1 (48)
3	-1 (8)	+1 (50)	-1 (48)
4	+1 (20)	+1 (50)	-1 (48)
5	-1 (8)	-1 (35)	+1 (102)
6	+1 (20)	-1 (35)	+1 (102)
7	-1 (8)	+1 (50)	+1 (102)
8	+1 (20)	+1 (50)	+1 (102)
9*	0 (14)	0 (42,5)	0 (75)
10*	0 (14)	0 (42,5)	0 (75)
11*	0 (14)	0 (42,5)	0 (75)
12	-1,68 (3,9)	0 (42,5)	0 (75)
13	+1,68 (24,1)	0 (42,5)	0 (75)
14	0 (14)	-1,68 (30)	0 (75)
15	0 (14)	+1,68 (55)	0 (75)
16	0 (14)	0 (42,5)	-1,68 (30)
17	0 (14)	0 (42,5)	+1,68 (120)

* Central points.

3. Results and Discussion

The application of pectinolytic enzymes in the umbu pulp aims especially the reduction of viscosity, in order to facilitate the pulp's processing. However, as a consequence, there is the release of colour compounds (pigments) from the vegetable's structure. Table 2 shows the experimental results of viscosity and colour of pulp with the application of pectinolytic enzyme varying in concentration, temperature and time on all three batches of umbu pulp.

Table 2. Viscosity and colour of umbu pulp (*L** and *b**), with application of pectinolytic enzyme in different concentrations, temperatures, times and pH values

Experiments	pH 2.3			pH 3.5			pH 4.5		
	Viscosity [cp]	*L**	b*	Viscosity [cp]	*L**	b*	Viscosity [cp]	*L**	b*
1	4.48	37.60	8.33	4.48	37.74	9.30	4.94	37.88	8.85
2	4.02	37.64	8.50	4.64	38.61	9.33	4.32	37.88	8.97
3	4.50	37.02	8.06	3.96	37.43	7.99	4.32	38.13	9.08
4	4.04	37.01	8.02	4.63	38.07	8.85	4.46	37.80	8.92
5	4.26	37.31	8.04	4.46	38.72	9.34	4.80	38.07	8.93
6	4.02	37.51	8.39	4.38	38.25	9.11	4.26	37.80	9.01
7	4.62	36.57	7.73	4.30	38.26	9.07	4.80	38.01	9.04
8	4.22	37.21	8.30	4.28	38.30	9.12	5.04	37.73	9.07
9	4.20	36.79	8.17	4.90	38.34	9.21	4.12	37.84	9.38
10	3.94	37.15	8.06	4.78	38.44	9.23	4.38	38.17	9.20
11	3.70	36.92	8.17	4.86	38.58	9.09	4.68	38.27	9.25
12	3.92	37.23	8.24	4.84	38.61	9.34	4.52	38.31	9.32
13	5.08	36.85	7.48	4.82	37.50	8.37	4.50	38.54	8.64
14	4.22	37.07	7.54	4.68	38.00	8.11	4.50	39.68	9.99
15	4.12	37.26	8.06	4.54	38.62	9.02	4.28	38.01	8.83
16	4.32	36.02	7.70	4.44	38.47	8.90	4.24	37.81	8.83
17	4.38	37.49	8.30	4.48	38.51	8.91	4.64	37.54	8.73

From the results obtained, initially, it was noted that the biggest loss of viscosity was observed in assay 11 with pH 2.3. Regarding colouring, the higher value of L* (luminosity) was obtained on assay 14, with pH 4.5, which also had the highest b* value. After extraction, the main part of the fruit juices is cloudy and shows high viscosity. UENEJO and PASTORE[22] report that, when adding pectinolytic enzymes in fruit pulps, there is a degradation of pectin and other components of high molecular weight. This causes a reduction in viscosity, allowing for a stronger separation between the liquid and solid phases, resulting in a high yielding of juices and a crystalline look to the product and reducing time of filtering in up to 50%.

Turbidity is caused mainly, due to positively charged proteins on the acid pH of juices, enveloped by negatively charged pectic substances. These substances remain suspended through the opposite charges on its surface, and the partial degradation of the pectin found in these particles allows the exposure of the protein nucleus. In turn, this causes an attraction between the nucleus of certain particles to the outer membrane of others, creating larger particles that precipitate. On the other hand, the pectins that are not involved with the protein particles are responsible for the viscosity in the juice, combined with hemicellulose. When these are hydrolyzed to smaller particles, they reduce viscosity significantly.[23]

With the assistance of the software Statistica 7.0, the results of the experiments were analyzed in order to verify the behavior of the studied variables, in order to optimize the process of applying pectinolytic enzymes on the umbu pulp. For the purposes of verifying which of the experiments were significant at a 95% confidence level, an ANOVA was performed and the determination coefficients were calculated. The values obtained are shown on Table 3.

Table 3. Determination coefficients and F calculated values obtained by ANOVA, for the models generated from the experimental results

	pH 2.3		pH 3.5		pH 4.5	
	R^2	$F_{calculated}$	R^2	$F_{calculated}$	R^2	$F_{calculated}$
Viscosity	0.93	10.83	0.26	0.27	0.78	2.80
*L**	0.22	0.22	0.88	5.84	0.46	0.29
*b**	0.63	1.34	0.77	2.74	0.42	0.55

$F_{critical\ (0.05;\ 1;\ g.l.\ resíduos)}$ = 3.68.

It was noted that only the models for pulp viscosity with pH 2.3 and L* with pH 3.5 were significant, as their F values were higher than the tabulated F (by ANOVA). This leads to the conclusion that the model is well adjusted to the observations and that its determination coefficients were reached. R^2 values were considered high, and the conclusion was that the variation of the results obtained could be explained by the adjusted model. Pareto graph and the Surface responses were generated for both assays, shown in Figures 1 and 2, respectively, for viscosity with pH 2.3.

Through the Pareto graph, it can be observed that, within the intervals of the studied independent variables, time and temperature are the ones which cause a significant effect (5%). UMSZA-GUEZ et al.[24] verified that the viscosity of the pulp of the caja-manga fruit is directly affected by the time and pectinolytic enzyme concentration, the study recommended ranges of the variables enzyme concentration, process temperature and incubation time, respectively, 0.042-0.068%, 47.0-49.0 ºC and 82-90 minutes. LEE et al.[25] used Pectinex Ultra SP-L to clarify banana juice, and noted that the temperature, enzymatic concentration and time of incubation influenced the juice clarification, where the optimum conditions were: 0.084% enzyme concentration, incubation temperature of 43.2 °C and incubation time of 80 minutes, a similar result was found by SHARMA et al for carrot juice. The results from those studies were similar to the results found, where the best conditions was in a low incubation temperature and intermediate incubation time.[26]

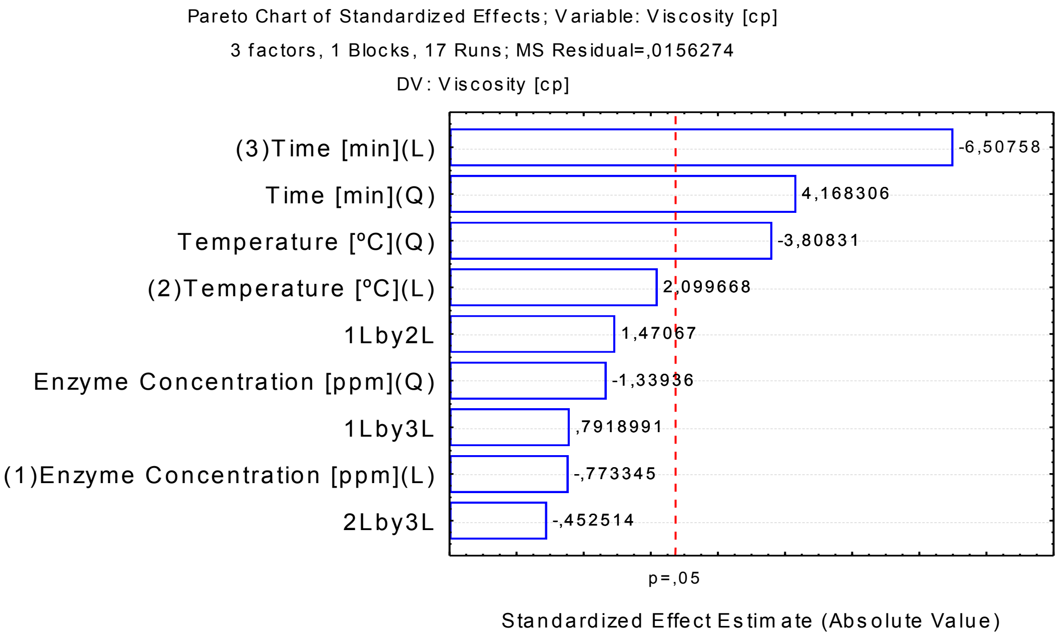

Figure 1. Pareto graph for the dependent variable viscosity at pH 2.3

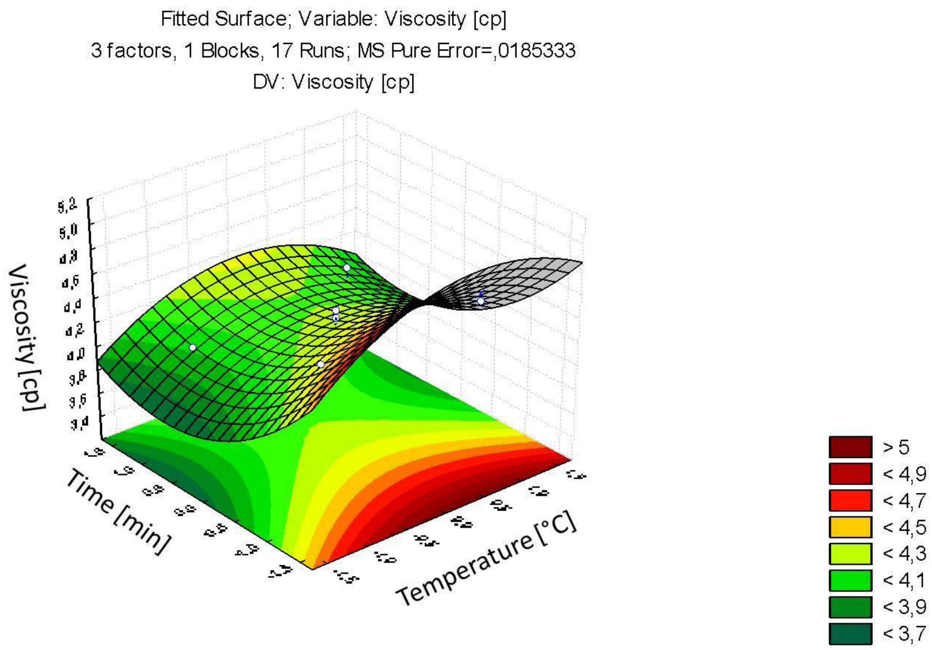

Figure 2. Response surface for dependent variable viscosity at pH 2.3

Kaur and Sharma[9] applied the pectinolytic enzyme in carrot juice and verified the decrease in viscosity in the juice after treatment in the established conditions. Analyzing the model obtained (Figure 2), it was observed that, in order to obtain a reduction in viscosity, the enzyme should be applied at a low temperature and for a long period of time (within the values used in this experiment). The encoding of the variables was made according to the following equation:

$$Z = 4{,}27 + 0{,}71x - 0{,}14x^2 - 0{,}22y + 0{,}15y^2$$

Where Z = viscosity (cp); x = time (min); y = temperature (◦C).

The influence of temperature on enzymatic activity involves two processes. First, the increase in temperature causes higher agitation of the molecules, and therefore higher possibilities of effect shock among them, increasing the speed of enzymatic reactions. However, this effect is observed at a temperature interval compatible with the maintenance of the enzyme's spatial structure. The other process relates to temperatures outside this interval. High temperatures cause the loss of the native structure of the enzymes, since it alters the chemical reactions that maintain their tri-dimensional structure.[27, 28]

The colour of a food product is one of the main attributes observed by the consumer. In the way, the monitoring of the colour is very important in the standardization of juices.[9] The Pareto graph and the response surface generated for L* with pH 3.5 are shown on Figures 3 and 4 respectively.

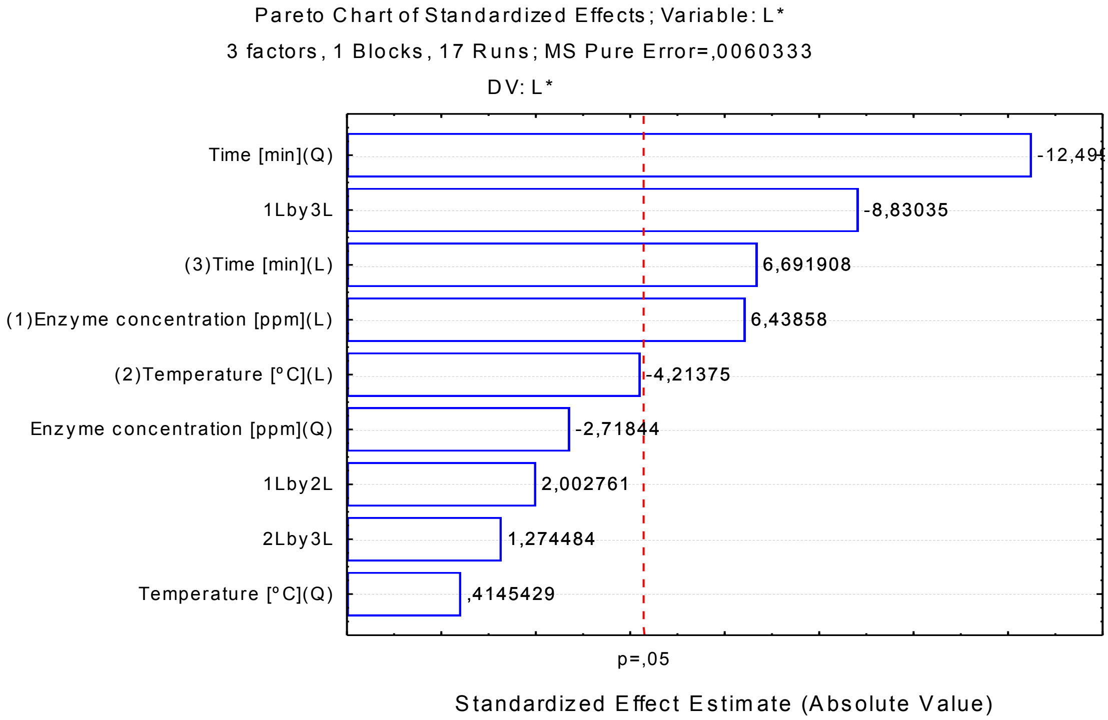

Figure 3. Pareto graph for the dependent variable *L** at pH 3.5

Through the obtained Pareto graph, it was observed that the variables time and enzymatic concentration (within the studied values) were those that had a significant effect (5%) on the pulp's luminosity. Umsza-Guez *et al.*[24] verified that the period of exposure to pectinolytic enzyme results in the increase of L* values. This was also found by Sin *et al.*[29] in the clarification of sapodilla juice. The encoding of the variables was made according to the following equation:

$$z = 38{,}54 - 0{,}08x + 0{,}009x^2 + 0{,}14y - 0{,}29y^2$$

Where Z = L*; x = time (min); y = enzyme concentration (ppm).

The response surface obtained indicates that the pulp reduces its luminosity with a shorter time and at lower enzyme concentration. This is to say that the clarification of the pulp is obtained at a higher enzymatic concentration and at intermediate time periods, within those values used in the experiment.

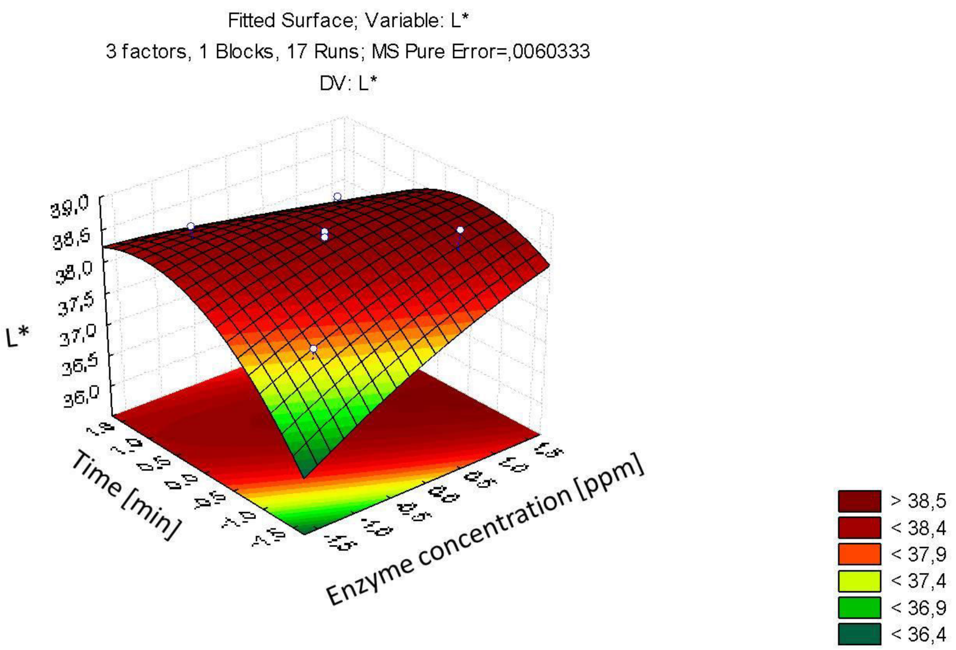

Figure 4. Response surface for dependent variable *L** at pH 3.5

The use of enzymes changed the umbu pulp studied, in their luminosity and viscosity. Many studies have been developed in order to evaluate the use of enzymes, Santos *et al.*,[30] evaluating the enzymatic treatment with pectinolytic enzymes in red 'araçá' (*Psidium cattleyanum Sabine*) juice found that the treatment favored the maintenance of the chemical composition of the natural juice, reduced viscosity and improved the extraction of phenolic compounds. The result was a final product with pronounced sensorial aspects and preservation of the nutritional characteristics of the fruit. Kempka *et al.*[31] evaluated the use of a commercial enzymatic preparation (Pectimax MA – Prozy) to clarify apple juice, and concluded that the temperature was the only significant variable, independently of the variety of apple used in the study. The enzymatic concentration was only significant in one of the varieties studied (Gala), having the yielding and juice clarification as responses. Therefore, the use of enzymes is very significant for juices industry, to increase the product quality.

4. Conclusion

This study indicates that higher enzyme concentrations result in a clarification of the pulp. The higher concentration also contributes to the viscosity reduction, if applied at low temperatures and low pH conditions. However, there was not found a best condition between treatments, it is suggested to be realized another design using parameters in higher enzyme concentration ranges and at lower temperatures, in order to reach the ideal point of optimal conditions for the application of the enzyme under study. From the results obtained in this experiment, it was observed that the pH interferes in the effect of application of pectinolytic enzymes in the umbu pulp, since different results were found. The models generated were only significant for the dependent variables viscosity and L* at pH 2.3 and 3.5 respectively. They were not significant for b*. Time and temperature were the significant variables for viscosity, with the lower viscosity reached at higher times and lower temperatures (within the limit tested). For luminosity, the significant variables were enzymatic concentration and time (within the values tested), with an increase in luminosity as time and enzyme concentration were increased. Therefore, it should consider the pH ratio, temperature and enzyme concentration to define the optimal conditions of the technical parameters to fruit pulp industrial application.

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