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Evaluation of Enzymatic Pretreatment of Passion Fruit Juice

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Yellow passion fruit juice is enjoyed worldwide due to its pleasant unique aroma and flavor. However, conventional stabilization processes induce general losses in the juice's original aroma and flavor. Membrane-based techniques are an alternative to obtain clarified and sterilized passion fruit juices. However, the high pectin and starch content makes the achievement of enhanced flow rates of permeate only possible after an enzymatic liquefaction of the juice. The scope of this work was to find the best condition for the enzymatic treatment of passion fruit in order to minimize the viscosity before the microfiltration process. The first step consisted on comparing the reduction of viscosity of the juice using different available commercial enzymes with pectinolityc, cellulase, and amylase activities. After the selection of the best enzyme-preparation, investigations were carried out in order to identify which were the significant variables for the enzymatic treatment. The studied variables were incubation time (from 30 to 120 min), enzyme concentration (from 0.025 to 5 mL/L), and temperature (from 25 to 50°C). Finally, the response surface methodology using a central composite planning was applied to predict the influence of input variables to achieve the highest viscosity reduction on the evaluated range of intervals. Results showed that enzyme preparations containing combined pectinolityc, cellulase, and amylase activities presented superior levels of viscosity reduction. Enzyme concentration and temperature were the two main constraints on the enzymatic liquefaction, whereas the incubation time did not have influence in the results after 30 minutes of reaction. The statistical analysis of the central composite planning showed that the best concentration and temperature values were 1mL/L and 50ºC, respectively, within the investigated interval.

1. Introduction

Yellow passion fruit (*Passiflora edulis*) is worldwide commercialized and largely cultivated in Brazil. This fruit is generally consumed as juice and its pleasant intensive aroma and flavor make it an attractive component for beverage and food industries (Vercelino-Alves et al., 2001).
Traditional stabilization methods, such as thermal pasteurization, are generally applied for fruit juice processing. However, heat processing results in change of yellow passion fruit aroma and flavor (Whitfield and Last, 1986).

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Membrane processes have been studied for clarification and concentration of fruit juices. This technology presents many advantages when compared to traditional processes, such as avoiding the use of additional agents for clarification and not using high temperatures for concentration and sterilization (Girard and Fukumoto, 2000).
However, fouling is a major constraint during separation using membranes. Its occurrence leads to a decline in membrane permeability.
Matta et al. (2000) indicated that the use of enzymatic pretreatment in fruit juice may increase membrane fluxes, since the presence of cell-wall polysaccharide compounds is the main cause of fouling occurrences in juice filtration (Vaillant et al., 1999). Ushikubo et al. (2007) showed the positive effect of enzymatic treatment for microfiltration of *Umbu* juice. Laorko et al. (2010) also applied an enzymatic treatment on apple juices before micro and ultrafiltration tests. Rai and De (2009) evaluated enzymatic treatments of *Mosambi* juice on membrane filtration performance showing that the centrifugation step also plays an important role in this process.
The scope of the present work was to evaluate different conditions of enzyme pretreatments for yellow passion fruit juice, aiming to minimize the juice viscosity for further membrane filtrations.

2. Materials and Methods

2.1 Passion fruit juice

The passion fruit juice was obtained after selection, washing, peel and seeds removal, using ripe fruit from plantations located in the region of Minas Gerais – Brazil. The juice was stored at -16ºC and it was defrosted to room temperature before use.

2.2 Analysis

Viscosities were measured by using a Brookfield LVDV-III digital rheometer at 25ºC. As some samples of pulpy juice presented a fast decantation behavior, the following procedure was used: samples were carefully agitated after the addition in the rheometer, and viscosities were measured at different shear rates (from 330 to 0 s^{-1}). All values expressed in this work were collected at 303 s^{-1} shear rate.

2.3 Enzymes and enzymatic treatment of juice

The enzymatic treatments were made in this work using different available commercial enzymes with different activities. Bacterial Amylase, Celluclast, and Pectinex 3X L were purchased from Novozymes, and Pectinase from *Aspergillus niger* was purchased from Sigma-Aldrich. Two enzymatic mixtures were also evaluated, by mixing equal quantities of different enzymes: Amylase + Celluclast+ Pectinex (mixture 1) and Pectinase + Amilase (mixture 2). Tests were conducted with these enzymes (pure and mixed) for 60 minutes in 250 mL conic flasks at constant agitation and temperature (200 rpm and 50ºC, respectively), using a concentration of 5 mL/L of each enzymatic preparations.
Using the most promising enzyme observed in preliminary tests, a 2^3 factorial planning was carried out in order to observe if the process variables reaction time, enzyme concentration, and temperature will influence the process. The experimental design and the statistic analysis were performed using the software STATISTICA 7.0. The significance of all terms in the statistic analyses were judged by computing the probability (P) of 0.05. A central composite planning was applied to find better

concentration and temperature values. The response function (y) was modeled as follows:

$y= b_0 +b_1X_1 + b_2X_2 +b_{12}X_1X_2 + b_{11}X_1^2 + b_{22}X_2^2$

where b_0 is a constant, b_1 and b_2 are the constant coefficients, b_{12} is the interaction coefficient, and b_{11} and b_{22} are the quadratic coefficients. X_1 and X_2 were the analyzed variables (enzyme concentration and temperature respectively). The response was measured in terms of viscosity reduction.

3. Results and Discussions

3.1 Rheological behavior

In terms of general rheological response, all the analyzed formulations have presented similar features. Figure 1 shows the shear stress versus shear rate for passion fruit pulp pure and treated with the proposed enzymes.

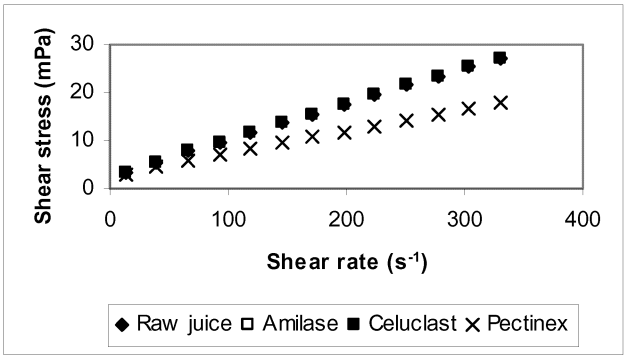


Figure 1. Rheological behavior of passion fruit pulp after enzyme treatments.

The passion fruit pulp obeys the model that describes a Bingham plastic fluid, exhibiting a linear shear-stress/shear-rate behavior after an initial shear-stress threshold. Between the analyzed enzymes, only the enzyme Pectnex showed significant viscosity reduction.

3.2 Selection of enzyme preparation

In order to evaluate the influence of varying enzymatic activity on the viscosity reduction, different enzyme preparations were tested (Table 1). All tests were made in triplicate. It was observed that the enzymatic preparations of Pectinex 3X L, Mixture 1, and Mixture 2 resulted in higher viscosity reductions. Taking availability and economic reasons into account, Pectinex 3X L, which contains pectinolityc, cellulase, and amylase activities, was chosen for use in subsequent experiments in this work.

3.3 Effect of enzyme concentration

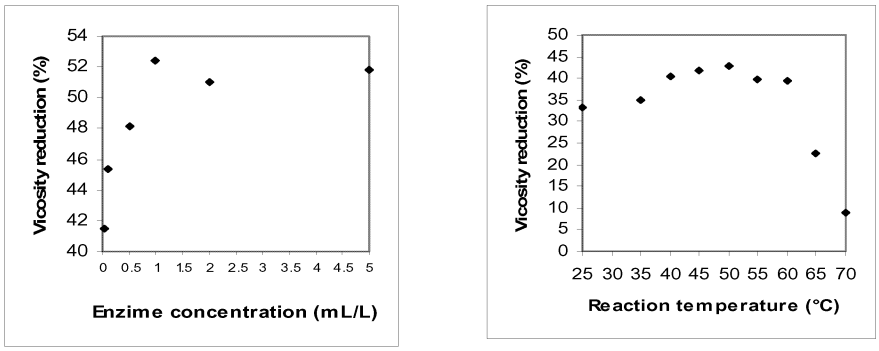
The effect of enzyme concentration was evaluated by treating the samples with different concentrations of Pectinex 3X L at 50ºC for 60 min. The results have shown that viscosity reduction is function of the concentration. However, runs with enzyme concentrations higher than 1 mL/L do not show increase in viscosity reduction (Figure 2a). Similar results were observed by Vaillant et al. (1999).

Table 1. Effect on enzymatic preparation on viscosity reduction of passion fruit juice

	Viscosity (cP)			Mean
Raw Juice	10.15	9.86	10.2	10.07
Amilase	8.59	8.38	8.02	8.33
Celuclast	9.91	8.56	8.89	9.12
Pectinex 3X L	4.96	4.81	4.85	4.87
Pectinase	8.57	9.56	7.98	8.70
Mixture 1	5.03	4.89	5.2	5.04
Mixture 2	5.95	6.23	5.76	5.98

3.4 Effect of temperature

The effect of temperature was evaluated by testing enzymatic reactions on several temperatures, using an enzymatic concentration of 1 mL/L at 60 min of incubation time. Results presented in Figure 2b shows that 50°C is the best temperature for enzymatic treatment of passion fruit juice. It is known that high temperatures deactivate the enzyme activities. These results suggest that the enzymatic reaction should not be carried out in temperatures higher than 50ºC.



(a) (b)

Figure 2. Effect of enzyme concentration and reaction temperature on viscosity reduction of passion fruit juice.

3.5 Effect of other variables and interactions

A factorial planning 2^3 was carried out to evaluate the effect of the single variables: incubation time, enzyme concentration, and temperature in the enzymatic treatment, and also the behavior of the interactions of them. Results presented in Table 2 show that viscosity reduction increases as temperature and enzyme concentration increases, within the analyzed interval.

Temperature and enzyme concentration have positive effects on viscosity reduction, and are significant at $P<0.05$. Standardized effects show that the temperature has a higher effect in comparison to the enzyme concentration. The time of incubation did not seem to be significant in the process, and neither any of the interactions between the observed

variables at P<0.05. Practical questions indicate the application of an incubation time equal to 60 min (Vaillant et al., 1999), in order to guarantee the complete reaction.

3.6 Central Composite Planning

Since factorial planning showed that temperature and enzyme concentration are the most important variables for viscosity reduction, a central composite planning was done in order to find the best condition for this treatment, as shown in Table 3. Table 4 shows that viscosity reduction is positively related to the linear effects of enzyme concentration and temperature. Both parameters are significant at P<0.05. Analysis of the central composite planning led to an optimum point of concentration and temperature of 1mL/L and 50ºC, respectively.

Table 2. 2^3 Factorial planning for enzymatic treatment of passion fruit juice

Run	Temperature (ºC)	Enzyme concentration (mL/L)	Time (min)	Viscosity (cP)	Viscosity Reduction (%)
1	25	0.1	30	6.86	31.88
2	50	0.1	30	5.38	46.57
3	25	1	30	6.51	35.35
4	50	1	30	4.99	50.45
5	25	0.1	120	6.82	32.30
6	50	0.1	120	5.48	45.56
7	25	1	120	5.70	43.40
8	50	1	120	4.68	53.53

Table 3: Central composite planning for enzymatic treatment of passion fruit juice

Run	Coded variables		Uncoded variables		Response	
	Enzyme concentration X_1	Temperature X_2	Enzyme concentration (mL/L)	Temperature (ºC)	Viscosity (cP)	Viscosity Reduction (%)
1	-1	-1	0.1	30	6.89	31.6
2	-1	1	0.1	50	5.59	44.52
3	1	-1	1	30	5.39	46.52
4	1	1	1	50	4.69	53.45
5	-1.15	0	0.0325	40	7.15	29.02
6	1.15	0	1.0675	40	5.36	46.78
7	0	-1.15	0.55	28.52	5.97	40.71
8	0	1.15	0.55	51.47	5.51	45.3
9	0	0	0.55	40	5.76	42.79
10	0	0	0.55	40	5.63	44.09
11	0	0	0.55	40	5.70	43.39

Table 4. Regression coefficient, R2 and coefficient values for viscosity reduction on enzymatic treatment of passion fruit juice

Regression coefficient	Viscosity (cP)	P
b_0	42.46	<0.0001
b_1	6.66	0.002
b_2	3.78	0.314
b_{11}	-1.86	0.025
b_{12}	-1.49	0.279
b_{22}	2.02	0.379
R^2	0.8982	

4. Conclusions

The passion fruit pulp showed to be a Bingham plastic fluid. Pectinex 3X L has proved to be the most efficient enzyme for viscosity reduction of passion fruit juice, among the commercial enzymes evaluated in this work. The 2^3 factorial planning showed that reaction time did not influence viscosity reduction of passion fruit juice, within the analyzed time intervals of 30 to 120 min. The central composite planning methodology suggests that the best conditions of temperature and enzyme concentration for the enzymatic treatment are 50ºC and 1 mL/L, respectively.

References

Girard, B. and Fukumoto, L.R., 2000, Membrane processing of fruit juices and beverages: a review, Critical Reviews in Food Science and Nutrition, 40, 91–157.

Laorko, A., Zhenyu, L., Tongchitpakdee, S., Chantachum, S. and Youravong, W., 2010, Effect of membrane property and operating conditions on phytochemical properties and permeate flux during clarification of pineapple juice, Journal of Food Engineering, 100, 514–521.

Matta, V.M., Cabral, L.M.C., Moretti, R.M., 2000, Clarification of acerola juice by enzymatic treatment and microfiltration, Alimentaria, 309, 127–130.

Rai, P. and De, S., 2009, Clarification of pectin-containing juice using ultrafiltration, Current Science, 96, 1361-1371.

Ushikubo, F. U., Watanabe, A. P. and Viotto A., 2007, Microfiltration of umbu (Spondias tuberosa Arr. Cam.) juice, Journal of Membrane Science, 288, 61–66.

Vaillant, F., Millan, P., O'Brien, G., Dornier, M., Decloux, M. and Reynes, M., 1999, Crossflow microfiltration of passion fruit juice after partial enzymatic liquefaction, Journal of Food Engineering, 42, 215–224.

Vercelino-Alves, R.M., Sarantopoulos, C.I.G.L., Segantini-Saron, E. and Bordin, M.R., 2001, Stability of fruit juice drinks in aseptic packages, Packaging Technology and Science, 14, 79–86.

Whitfield, F.B. and Last, J.H.,1986, The flavour of the passionfruit - a review. In: Brunke, E.J. (ed.) Progress in essential oil research. Berlin: Walter de Gruyter e Co., 3-48.