ISSN 0101-2061 (Print)
ISSN 1678-457X (Online)
Food Science and Technology

DOI: https://doi.org/10.1590/fst.11221

Use of encapsulated commercial enzyme in the hydrolysis optimization of cagaita pulp (*Eugenia dysenterica* DC)

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Abstract

Cagaita (*Eugenia dysenterica* DC) is a Brazilian cerrado fruit with great economic potential and can be consumed *in natura* or as processed products like juices and pulps. The search for products with lower nutritional and sensorial changes led to the non-thermal techniques development where membrane processes stand out. The use of immobilized pectinolytic enzymes to reduce juices and pulps turbidity and viscosity has advantages over free enzymes such as enzyme reduction costs and reuse. The objective was the hydrolysis optimization of cagaita pulp with encapsulated pectinase, evaluation of reuse in cycles and application in microfiltration (MF). The free commercial enzymes and encapsulated activity in calcium alginate in pulp was evaluated, viscosity and turbidity reduction. The optimum hydrolysis with encapsulated enzymes conditions were temperature (30 °C), without stirring, enzymatic concentration (570 μL/L), considering clarity increment and viscosity reduction. After 8 cycles, encapsulated enzymes maintained 30% of its activity in reducing viscosity and reuse possibility. Microfiltration flow rate of hydrolyzed pulp with encapsulated enzymes was 13.4% higher than the nonhydrolyzed, indicating that enzymatic hydrolysis was efficient in time reduction. Encapsulated enzymes can be applied in juices and pulps as a pre-process for increasing the permeate flows, reducing operational and input costs.

Keywords: *Eugenia dysenterica* DC; cagaita; microfiltration; microencapsulation; alginate.

Practical Application: The results obtained in the study provided that the use of encapsulated enzymes minimize enzymes costs in the fruit juices industry.

1 Introduction

The Cerrado region and the Brazilian Pantanal present a great fruit species variety, still underutilized by local communities for the lack of scientific knowledge and incentive for its commercialization. The cagaiteira (*Eugenia dysenterica* DC.) belongs to the *Myrtaceae* family and is a native species of the Cerrado with great potential of introduction to the crop, for producing fruits appreciated, both for *in natura* consumption and in the processed forms as juices, fermented beverages (Figueiredo et al., 2019), liqueur, jelly and sweets among others. Aditionally the seeds, peels and pulp of cagaita present antioxidant capacity for the elaboration of nutritional supplements (Kaur & Kapoor, 2001; Roesler et al., 2007; Alves et al, 2017; Santos et al., 2018).

The main methods used for fruit juices and pulps preservation are the pasteurization and other thermal processes like UHT (Ultra High Temperature). These methods, while guaranteeing microbiological quality, cause undesired sensory and nutritional changes. The MF, ultrafiltration (UF) and reverse osmosis (RO) have been used in large scale and have been successfully applied to some highly thermo-sensitive juices, resulting in a clarified and microbiologically safe product that preserves most of the original fruit aroma (Vaillant et al., 1999), (Rodrigues et al., 2003) (Vieira et al., 2006) and Carvalho & Silva (2010).

The limitation to membrane processes is found in the juices rheological properties where high-viscosity ones increase the process time requiring higher working pressure, which demands a higher feed pump effort and a higher wear of the membranes (Koblitz, 2008).

The most widespread method for fruit juices viscosity reduction is enzymes use. The application of pectinolytic and amylolytic enzymes leads to pectin and starch compounds reduction that cause viscosity, increasing the yield in a short processing time and reducing residues produced (Sarioiğlu et al., 2001; Koblitz, 2008; Silva et al, 2008a; Carneiro et al., 2020; Antigo et al., 2018; Cardoso et al., 2019).

On the other hand, the high cost of enzymes isolation and purification, the instability of their structures once isolated from their natural environment, their susceptibility to the process conditions and the presence of inhibitory compounds, even in low concentrations, can prevent the application of these biocatalysts in food juices industry. One of the widely used alternatives to overcome these limitations is enzyme immobilization. In this

Received 25 Feb., 2021
Accepted 27 Feb., 2021
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way the enzyme structure is stabilized, making it more resistant to medium reaction. In addition, immobilization allows easy enzyme recovery and its reuse. The use of immobilized enzymes in juices clarification can lead to an increase in its activity, as well as the costs reduction, making its use possible (Adlercreutz, 1993; Vitolo, 2001). This work aimed the production of encapsulated pectinolitic enzymes in calcium alginate, to apply as a previous step in cagaita pulp clarification by microfiltration (MF) and its reutilization.

2 Materials and methods

2.1 Enzymes and cagaita pulp

Commercial pectinolytic enzyme Pectinex® Ultra Clear (*Aspergillus acuteatus*, *Aspergillus niger*) (Novozymes Latin America Ltda) was used for the experiments. The cagaita pulp was obtained from ripe fruits, harvested in January 2015 in the Chapada Gaúcha city, located in Minas Gerais northern state, Brazil, and kept under freezing at -18 °C until the experiments and analyzes.

2.2 Effect of the enzymatic activity on pulp clarity

It was performed by measuring the transmittance of the cagaita pulp in a spectrophotometer at 540 nm after centrifugation at 3000 rpm for 10 min. (Singh et al., 2012). The analyzes were performed in triplicates.

2.3 Effect of enzymatic activity on viscosity

The viscosity determination (Ubbehlode type II capillary viscometer - LAUDA) was used in a thermostatic bath at 25 °C (Bermejo-Prada et al., 2015). The analyzes were performed in triplicates.

2.4 Enzymes encapsulation in calcium alginate

The capsules were obtained by dropping sodium alginate and the buffer solution containing the enzymes into a calcium chloride solution using a peristaltic pump Cole Parmer - Model 7523-80, Masterflex YZ-06475-14 L/S hose 14 and needles of 0.80 x 25 mm, 0.55 x 20 mm, 0.45 x 13 mm and 0.3 x 13 mm.

Equal volumes of sodium alginate and commercial enzymes diluted in citrate-phosphate buffer (pH 4.0) were homogenized at 150 rpm for 5 min in a Fisatom 713D equipment. Two mL of sodium chloride solution and enzyme were dropped at a flow rate of 2 mL/min. in 20 mL of calcium chloride solution. The capsules formed were submerged in calcium chloride solution for 30, 60 or 90 minutes at 4 °C. The capsules were washed in deionized water and stored in citrate-phosphate buffer solution pH 4.0. The calcium chloride solution and the deionized water used during the capsule formation process were stored for protein content analysis and pectinase activity (Rehman et al., 2013).

2.5 Experimental Design for optimization of the pulp hydrolysis parameters with free and encapsulated enzymes

In order to determine the conditions to be used in the reuse of encapsulated pectinase and in cagaita pulp prior MF, the optimization of temperature, enzyme concentration and stirring on the responses: increasing the clarity and the pulp viscosity reduction. The optimization was also performed to compare the profile of these variables for the free and encapsulated enzymes.

For optimization, a central rotational planning was used in 5 levels of variation with 4 repetitions of the central point using the software STATISTICA 7.0.

Free enzyme

In Table 1 are presented the parameters with their respective values used in the experimental planning of pulp hydrolysis for the free enzyme.

The conditions used in the analysis of increase of clarity and viscosity reduction for the free enzyme were based on the worksheet generated by the software STATISTICA 7.0 according to Table 2.

Encapsulated Enzyme

Table 3 presented the parameters with their respective values used in experimental design of the cagaita pulp hydrolysis for encapsulated enzyme. The enzyme concentrations are slightly different from those for free enzyme. Activities below 90.7 μL/L in the preliminary tests were null.

The conditions used in the analysis clarity increase and viscosity for free enzyme reduction were based on the spreadsheet generated by the software according to Table 4.

2.6 Evaluation of the reuse of the encapsulated enzymes

It was evaluated by determining the enzyme residual activity after 7 replicates of each cycle of its reuse.

2.7 Microfiltration Process (MF)

Hydraulic permeability

For evaluation of the state and integrity of membrane after cleaning, the hydraulic permeability was measured. The membrane hydraulic permeability was measured with distilled water according to the following Equation 1:

$$\text{Hydraulic permeability} = v/(A \cdot P) \quad (1)$$

Where: v is the obtained flow (L / h). P is the pressure (bar) and A is the membrane surface area (m^2).

Table 1. Parameters and values used for the optimization of pulp hydrolysis using free enzyme.

Parameters	-α	-1	0	1	+α
Temperature (°C)	30	37.1	47.5	57.9	65
Enzime Conc. (μL/L)	40	185.7	400	614.3	760
Stirring (rpm)	0	30	60	90	120

Table 2. Experimental design for optimization of the pulp hydrolysis with free enzyme.

Assays	Temperature (°C)	Pectinase Concentration (μL/L)	Stirring (rpm)	Temperature (°C)	Pectinase Concentration (μL/L)	Stirring (rpm)
1	-1,00	-1.00	-1.00	37.1	185.7	24.3
2	-1.00	-1.00	1.00	37.1	185.7	95.7
3	-1.00	1.00	-1.00	37.1	614.3	24.3
4	-1.00	1.00	1.00	37.1	614.3	95.7
5	1.00	-1.00	-1.00	57.9	185.7	24.3
6	1.00	-1.00	1.00	57.9	185.7	95.7
7	1.00	1.00	-1.00	57.9	614.3	24.3
8	1.00	1.00	1.00	57.9	614.3	95.7
9	-1.68	0.00	0.00	30.0	400.0	60.0
10	1.68	0.00	0.00	65.0	400.0	60.0
11	0.00	-1.68	0.00	47.5	40.0	60.0
12	0.00	1.68	0.00	47.5	760.0	60.0
13	0.00	0.00	-1.68	47.5	400.0	0.0
14	0.00	0.00	1.68	47.5	400.0	120.0
15 (C)	0.00	0.00	0.00	47.5	400.0	60.0
16 (C)	0.00	0.00	0.00	47.5	400.0	60.0
17 (C)	0.00	0.00	0.00	47.5	400.0	60.0
18 (C)	0.00	0.00	0.00	47.5	400.0	60.0

Table 3. Parameters and values for the optimization of cagaita pulp hydrolysis with encapsulated enzymes.

Parameters	-α	-1	0	1	+α
Temperature (°C)	30	37.1	47.5	57.9	65
Enzime Conc. (μL/L)	90.7	257.4	502.5	747.6	914.2
Stirring (rpm)	0	30	60	90	120

Microfiltration of the cagaita pulp

To evaluate the efficiency of the enzymatic hydrolysis, the filtration of cagaita pulp was carried out in a KOCH Industries' PROTOSEP IV system with PENTACOR MFK 617 tubular polyethersulfone membrane (Koch Membrane Systems Inc., Massachusetts) with 5 channels and a mean pore diameter of 0.3μm and surface area of 0.05 m^2.

Cagaita pulp permeate flux

To evaluate the efficiency of the cagaita pulp enzymatic hydrolysis, the permeate flux was determined under a pressure of 0.5 bar. During the MF the permeate pulp flow was obtained from the Equation 2:

$$\text{Flow} = v / A \quad (2)$$

Where: v is the obtained flow (L / h) and A is the surface area of the membrane (m^2).

2.8 Statistical analysis

The variance analysis was performed with the GraphPad Prism software using Tukey's test as a *post*-test with confidence level of $p < 0.05$. The multivariate statistical methodology was also performed. For the preliminary tests, a complete Factorial Design (2^n) was used in 3 levels of variation with 4 repetitions of the central point. To optimize the parameters, central rotation planning was used in 5 levels of variation with 4 repetitions of the central point and, for the generation of the test sheets and the evaluation of parameters significance, the STATISTICA 7.0 software was applied (Rodrigues & Iemma, 2005).

3 Results and Discussion

3.1 Optimization of cagaita pulp hydrolysis with free and encapsulated enzymes

Tables 5 and 6 present the results from the optimization analysis of the cagaita pulp hydrolysis with free and encapsulated enzymes, respectively.

Clarity increment

Use of the free enzymes

The variance analysis to verify the clarity increment with free commercial enzyme was significant ($p \leq 0.05$). Only the variables stirring (quadratic effect) and the interaction between temperature and enzymatic concentration were not significant at $p \leq 0.05$ and the determination coefficient (R^2) was 0.75. The Equation 3 for the regression model to clarity increment was:

$$\begin{aligned}\text{Clarity increment } (\%) &= 267.05 - 10.3782X_1 \\ &+ 0.1063X_1^2 + 0.1082X_2 - 0.00014X_2^2 \\ &+ 0.6283X_3 - 0.0130X_1X_3 + 0.00075X_2X_3\end{aligned} \quad (3)$$

Table 4. Experimental Design for optimization of the cagaita pulp hydrolysis with encapsulated enzymes.

Assay	Temperature (°C)	Pectinase Concentration (μL/L)	Stirring (rpm)	Temperature (°C)	Pectinase Concentration (μL/L)	Stirring (rpm)
1	-1.00	-1.00	-1.00	37.1	257.4	24.3
2	-1.00	-1.00	1.00	37.1	747.6	24.3
3	-1.00	1.00	-1.00	37.1	257.4	95.7
4	-1.00	1.00	1.00	37.1	747.6	95.7
5	1.00	-1.00	-1.00	57.9	257.4	24.3
6	1.00	-1.00	1.00	57.9	747.6	24.3
7	1.00	1.00	-1.00	57.9	257.4	95.7
8	1.00	1.00	1.00	57.9	747.6	95.7
9	-1.68	0.00	0.00	30.0	502.5	60.0
10	1.68	0.00	0.00	65.0	502.5	60.0
11	0.00	-1.68	0.00	47.5	502.5	0.0
12	0.00	1.68	0.00	47.5	502.5	120.0
13	0.00	0.00	-1.68	47.5	90.7	60.0
14	0.00	0.00	1.68	47.5	914.2	60.0
15 (C)	0.00	0.00	0.00	47.5	502.5	60.0
16 (C)	0.00	0.00	0.00	47.5	502.5	60.0
17 (C)	0.00	0.00	0.00	47.5	502.5	60.0
18 (C)	0.00	0.00	0.00	47.5	502.5	60.0

Independent variables in coded and uncoded values.

Table 5. Cagaita pulp hydrolysis optimization with free Pectinex® Ultra Clear.

Assay	Temperature (°C)	Enzyme Concentration (U/L)	Stirring (rpm)	Clarity increment (%)	Viscosity reduction (%)
1	37.1	185.7	24.3	35.27	7.42
2	37.1	185.7	95.7	72.95	14.88
3	37.1	614.2	24.3	49.76	11.08
4	37.1	614.2	95.7	94.20	16.91
5	57.9	185.7	24.3	44.93	5.40
6	57.9	185.7	95.7	46.86	10.96
7	57.9	614.2	24.3	52.66	3.67
8	57.9	614.2	95.7	94.20	12.71
9	30.0	400.0	60.0	123.19	15.99
10	65.0	400.0	60.0	42.03	10.07
11	47.5	40.0	60.0	22.71	9.53
12	47.5	760.0	60.0	41.06	15.27
13	47.5	400.0	0.0	45.16	11.59
14	47.5	400.0	120.0	61.29	14.33
15	47.5	400.0	60.0	55.56	14.35
16	47.5	400.0	60.0	50.72	14.97
17	47.5	400.0	60.0	49.76	15.09
18	47.5	400.0	60.0	53.62	15.99

Where X_1: temperature (°C). X_2: enzyme concentration (μL/L). X_3: stirring (rpm). X_1X_3: interaction between temperature and stirring and X_2X_3: interaction between enzyme concentration and stirring.

The enzyme activity was higher at lower temperatures decreasing between 40 and 55 °C which was the optimum range of pectinase activity (Figure 1).

An enzyme activity accentuated could lead to high hydrolysis of the pectin molecules that surround the positive charge protein nucleus of the plant cytoplasm causing repulsion between the particles, maintaining the pulp turbidity and the decrease of the clarity (Koblitz, 2008). From 55 °C, the Pectinex® Ultra Clear activity increased again.

The same behavior was observed by Télesphore & He (2009), hydrolyzing passion fruit pulp (*Passiflora edulis*) with Pectinex Ultra SP-L, observing an increase in pulp turbidity between 35 and 45 °C and decrease thereafter, while Rai et al. (2004) reported that with *Aspergillus niger* pectinase, the clarity increased with temperature increasement (32-49 °C) during the 'Valencia' orange juice (*Citrus sinensis* (L.) Osbeck) hydrolysis.

Table 6. Cagaita pulp hydrolysis optimization with the encapsulated enzymes.

Assay	Temperature (°C)	Enzyme conc. (μL/L)	Stirring (rpm)	Clarity increment (%)	Viscosity reduction (%)
1	37.1	257.4	24.3	9.59	12.63
2	37.1	747.6	24.3	38.36	15.34
3	37.1	257.4	95.7	34.78	14.67
4	37.1	747.6	95.7	61.96	19.77
5	57.9	257.4	24.3	25.00	13.06
6	57.9	747.6	24.3	25.00	15.80
7	57.9	257.4	95.7	0.89	11.06
8	57.9	747.6	95.7	3.57	15.32
9	30.0	502.5	60.0	48.91	15.42
10	65.0	502.5	60.0	23.21	8.80
11	47.5	502.5	0.0	12.59	21.85
12	47.5	502.5	120.0	1.48	20.79
13	47.5	90.7	60.0	0.74	17.23
14	47.5	914.2	60.0	38.52	20.76
15	47.5	502.5	60.0	6.47	29.23
16	47.5	502.5	60.0	2.35	32.85
17	47.5	502.5	60.0	1.18	29.33
18	47.5	502.5	60.0	1.76	30.77

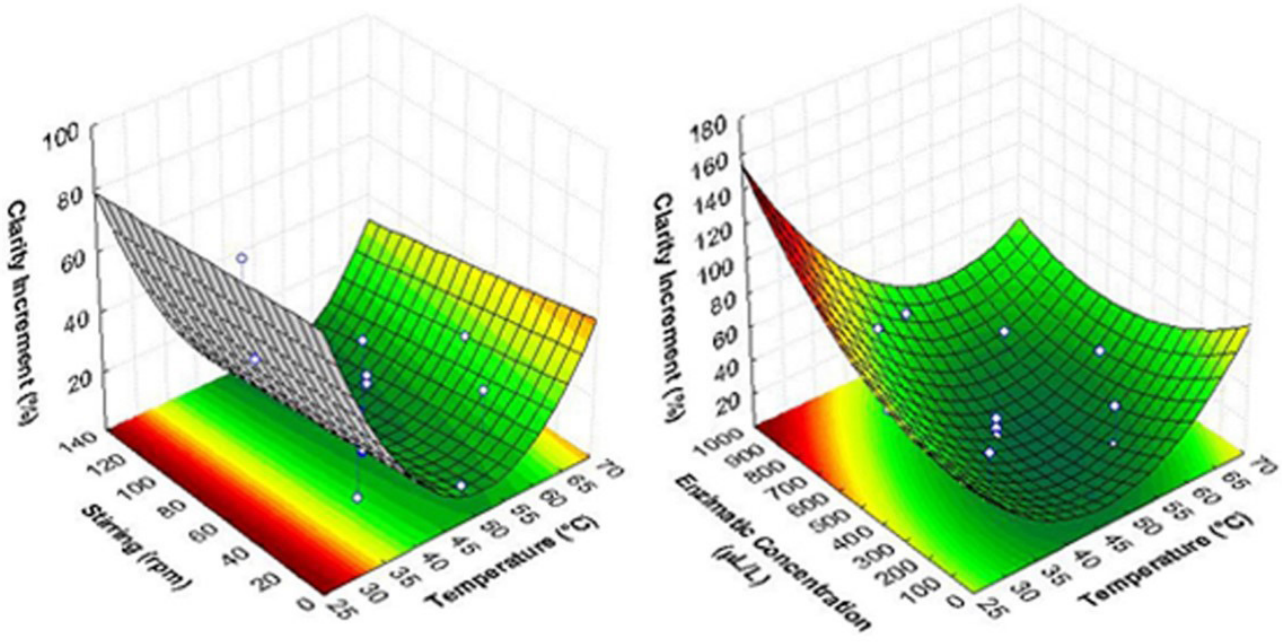

Figure 1. Response surface for the clarity increment for the enzyme as a function of temperature and enzymatic concentration and enzymatic concentration and stirring.

The commercial enzyme concentration presented a quadratic pattern with maximum activity between 500 and 700 μL/L depending on the conditions used (Figure 1). Very high concentrations could also lead to a very marked hydrolysis of the pectin molecules increasing the clarity. Télesphore & He (2009) reported a turbidity increasing in passion fruit juice according to the concentration of commercial enzyme increasing. Higher stirring led to an enzyme activity increase. The activity increased 105.0% when the stirring increased from 0 to 120 rpm.

Encapsulated commercial enzyme

The variance analysis showed significant in both levels tested ($p \leq 0.05$ and $p \leq 0.01$). Only the stirring (linear and quadratic effects) and the interaction between stirring and enzymatic concentration variables were not significant at $p \leq 0.05$ and the determination coefficient (R^2) was 0.96.

The Equation 4, for the regression model in the clarity increment was:

$$\text{Clarity increment } (\%) = 242.60 - 9.9771X_1 + 0.1099X_1^2 + 0.0583X_2 + 0.0001X_2^2 - 0.0015X_1X_2 - 0.0026X_1X_3 \quad (4)$$

Where X_1: temperature (°C). X_2: enzymatic concentration (rpm). X_1X_2 = interaction between temperature and enzymatic concentration and, X_1X_3: interaction between temperature and stirring.

The enzyme activity reduced only 8.5% with stirring presenting an increasing from 0 to 120 rpm, indicating that its variation little contributed to the clarity increase as occurred with the free enzyme (Figure 2).

The enzyme activity was higher at lower temperatures decreasing 84.5% of its value at 52.5 °C and increasing to 65 °C (Figure 2). The optimum temperature for encapsulated enzyme activity was between 40 and 50 °C. The same behavior was observed for free enzyme. Higher enzyme concentrations (above 700 mL/L) resulted in higher activity (Figure 2). Possibly higher concentrations than those studied can reach the optimum point of concentration for the clarity increment.

Viscosity reduction

Free commercial enzyme

The variance analysis of the viscosity reduction for free commercial enzyme was significant for both levels tested ($p \leq 0.05$ and $p \leq 0.01$). The interactions between independent variables (X_1X_2, X_1X_2 and X_3X_4) were not significant at $p \leq 0.05$ and the determination coefficient (R^2) was 0.79.

The Equation 5, obtained for the regression model for viscosity reduction was:

$$\text{Viscosity reduction } (\%) = -15.81 + 0.8815X_1 - 0.0113X_1^2 + 0.0305X_2 - 0.00003X_2^2 + 0.1846X_3 - 0.0010X_3^2 \quad (5)$$

Where: X_1: Temperature (°C), X_2: enzymatic concentration (μL/L) and X_3: stirring (rpm).

All the independent variables presented a quadratic pattern (Figure 3). The enzyme activity increased to 38.9 °C (5.5%) and decreased from this point 44.2% at the temperature of 65 °C. Enzyme activity increased 99.0% when stirring rose from 0 to 93.9 rpm. Slower stirring could decrease the contact between enzyme and substrate as well as in higher stirring there could be the mechanical enzyme denaturation.

The optimum conditions for maximum viscosity reduction were 38.9 °C, concentration of 483.1 μL/L and stirring of 93.9 rpm. The optimum temperature is in accordance with other authors (Sagu et al., 2014; Lee et al., 2006; Rai et al., 2004 and Silva, 2008b). Comparing the results of viscosity reduction with those of clarity increment these conditions parameters can be considered optimal values for the cagaita pulp hydrolysis with the free enzyme.

Lee et al. (2006) optimized the conditions of enzymatic concentration, temperature and time for clarification of banana juice (*Musa sapientum*) cv Berangan using the surface response analysis. The viscosity decreased to a certain point at lower temperature and time, with which it increased slightly.

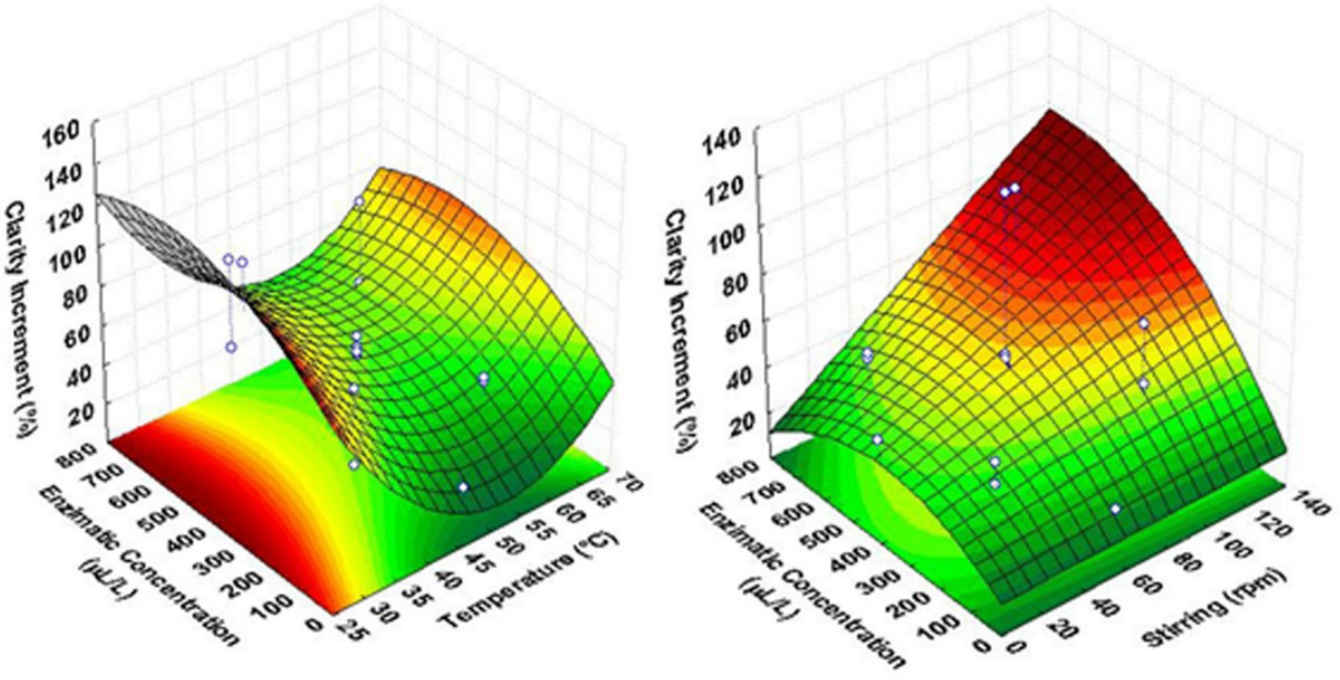

Figure 2. Response surface and response profile for the clarity increment of the encapsulated enzyme as a function of the temperature and stirring and temperature and enzymatic concentration.

The optimum conditions for viscosity reduction in banana juice were enzymatic concentration of 0.098%, temperature of 42.9 °C and time of 75.24 minutes.

Sagu et al. (2014) studied the effects of enzyme concentration parameters on temperature and time for banana pulp (*Musa acuminata*) hydrolysis. The results showed that the viscosity was reduced with the increasing time and enzyme concentration. The banana pulp viscosity decreased with temperatures between 35 and 40 °C. According to the authors, higher temperatures may lead to the formation of pectin gels by increasing the solution viscosity. The optimum conditions for all dependent variables tested (viscosity, clarity, alcohol soluble solids, total polyphenols and protein concentration) were: temperature of 33 °C, incubation time of 108 min and pectinase concentration of 0.03%.

Rai et al. (2004) analyzed the enzymatic concentration, time and temperature in the Valencia orange juice (*Citrus sinensis* (L.) Osbeck) hydrolysis using the response surface analysis. The juice viscosity decreased linearly with increasing time and the enzymatic concentration, at a constant temperature. The optimum/best conditions were 0.0004% of enzymatic concentration, time of 99.27 min and temperature of 41.89 °C.

Encapsulated commercial enzymes

The viscosity reduction was significant for both levels ($p \leq 0.05$ and $p \leq 0.01$) with the enzyme commercial encapsulated. The variables temperature (linear effect), stirring (linear effect) and the interactions between independent variables were not significant at $p \leq 0.05$ and the coefficient of determination (R^2) was 0.92, value considered high.

The Equation 6, obtained for the regression model for viscosity reduction was:

$$\text{Viscosity reduction } (\%) = 11.75 - 0.0020X_1^2 - 0.00011X_2^2 + 0.0520X_3 - 0.00005X_3^2 \quad (6)$$

Where X_1: temperature (°C). X_2: stirring (rpm) and X_3: enzyme concentration (μL/L).

The viscosity reduction was higher at lower temperatures since the increase in temperature from 30 to 65 °C led to an activity reduction of 26.8% (Figure 4). The temperature viscosity reduction pattern of the encapsulated enzymes was different from that found for free enzyme. Possibly, due to the encapsulation process. However, it was similar to that found in the clarity increment for the encapsulated enzyme (Figure 2).

The difference in viscosity reduction between the static reaction (0 rpm) and the highest stirring one (120 rpm) was only 6.2% (Figure 4). Due to the low coefficient attributed to the stirring variable (X_2) in Equation 5, it is possible to observe that, although significant, its variation had low influence on the viscosity reduction. This behavior was similar to that found in the of clarity increment with the encapsulated enzyme (Figure 2).

The enzyme concentration presented a similar pattern to that of the free enzyme (Figure 3). The optimum enzyme concentration of the encapsulated one was 570.2 μL/L, higher than that found for free enzyme (483.1 μL/L).

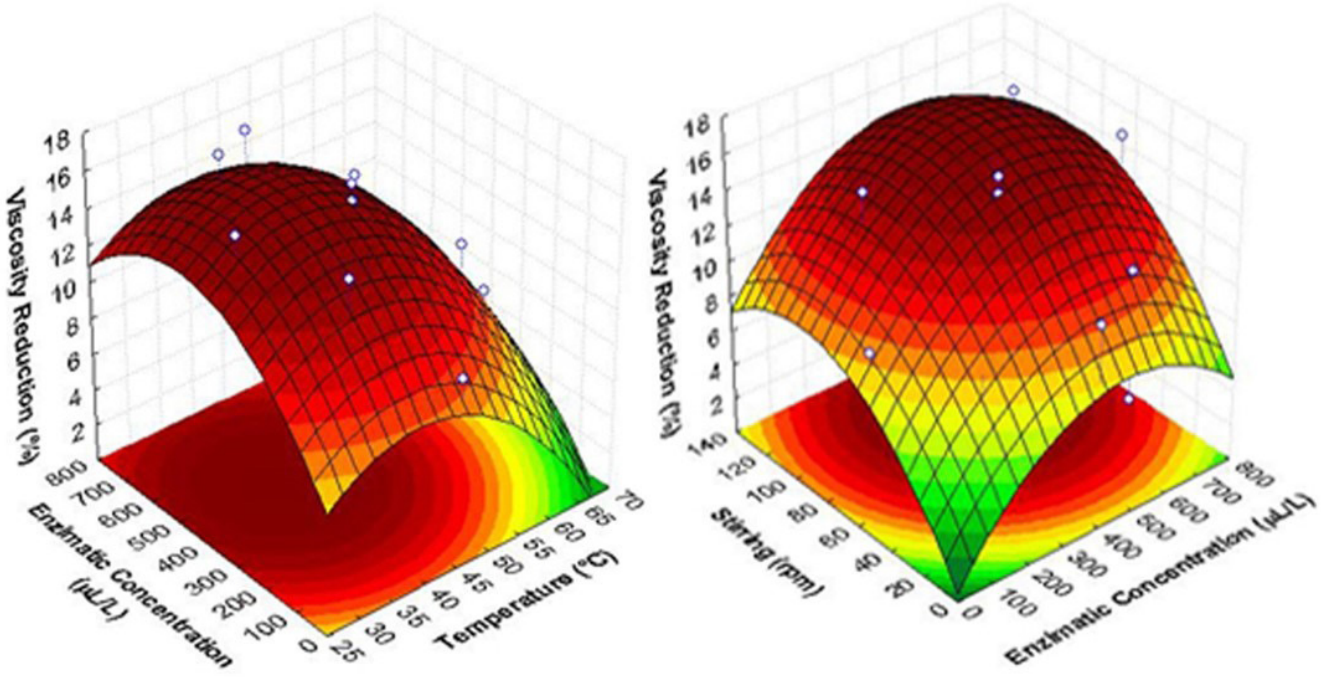

Figure 3. Response surface and response profile for the viscosity reduction of free pectinase as a function of temperature and enzymatic concentration and temperature and stirring.

Comparing the results of the clarity increment and viscosity reductions, the optimal conditions for cagaita pulp hydrolysis were: temperature (30 °C) without stirring which were the best conditions for the two tests and the enzymatic concentration of 570 μL/L, concentration is optimal for viscosity reduction that has greater relevance in MF efficiency. These values were used in the validation of the equation obtained for the regression model. The difference of the calculated result for the experimental one was of 0.64%.

3.2 Reuse of the encapsulated commercial enzyme

The enzyme stability was assessed by reusing the encapsulated enzyme, which was evaluated by the clarity increment and viscosity reduction of the cagaita pulp analysis. The clarity increment did not differ significantly ($p \leq 0.05$) in the eight cycles tested. Most likely, the loss of enzyme to the medium during the eight cycles studied did not influence significantly in a change in the clarity increment of the cagaita pulp.

The encapsulated commercial enzyme maintained 52% of the initial viscosity reduction activity in the second cycle, 45% in the third and around 30% by the end of the eighth cycle. The decrease in activity was accentuated in the second cycle but, from this point there was no significant difference between two subsequent cycles, and the final residual activity (30%) was more elevated than other several test found in the literature (Rehman et al., 2013; Anwar et al, 2009; Kumar et al., 2006).

The activity reduction is probably due to the enzyme loss from the capsule to the medium during its use. Although the sodium alginate and calcium chloride concentrations were not significant in the capsule formation, the use of these parameters at higher concentrations could lead to a reduction in pore size and, consequently, a lower loss of enzyme to the medium (Wu et al., 2010; Anwar et al., 2009; Arya & Srivastava, 2006).

Differently, Rehman et al. (2015) analyzed the pectinase pattern produced by *Bacillus licheniformis* KIBGE-IB21 encapsulated in calcium alginate. The encapsulated pectinase maintained 80% of its activity in the second cycle, 65% in the third cycle and 9% at the end of the seven cycles. According to them, the decrease in activity could be related to the enzyme loss during the capsules washing with deionized water between the cycles or the change of conformation of the enzyme by its repeated use.

Similarly, Anwar et al. (2009) monitored the activity of the *Bacillus subtilis* protease KIBGE-HAS encapsulated in calcium alginate for 4 cycles of reuse. The enzyme showed 80% of the activity in the second cycle, 35% in the third and, totally lost the activity in the fourth cycle. They attributed the activity reduction to enzyme loss during the capsules washing between the cycles.

Additionally, Ganaie et al. (2014) analyzed the fructosyltransferase activity of *Aspergillus flavus* NFCCI 2364 mycelium fragment encapsulated with chitosan and calcium alginate for 15 cycles. The encapsulated enzyme reduced its activity from the 4th cycle of reuse. The enzyme encapsulated in calcium alginate maintained its initial activity during the first 7 cycles.

Kumar et al. (2006) studied the residual activity of encapsulated α-amylase in calcium alginate capsules of various sizes during six cycles of reuse. They reported that reusability stability is dependent on capsule size and when smaller than 1 mm were more stable and maintained 70% of the initial activity at the end of the 6 cycles. Capsules larger than 1 mm ended the sixth cycle

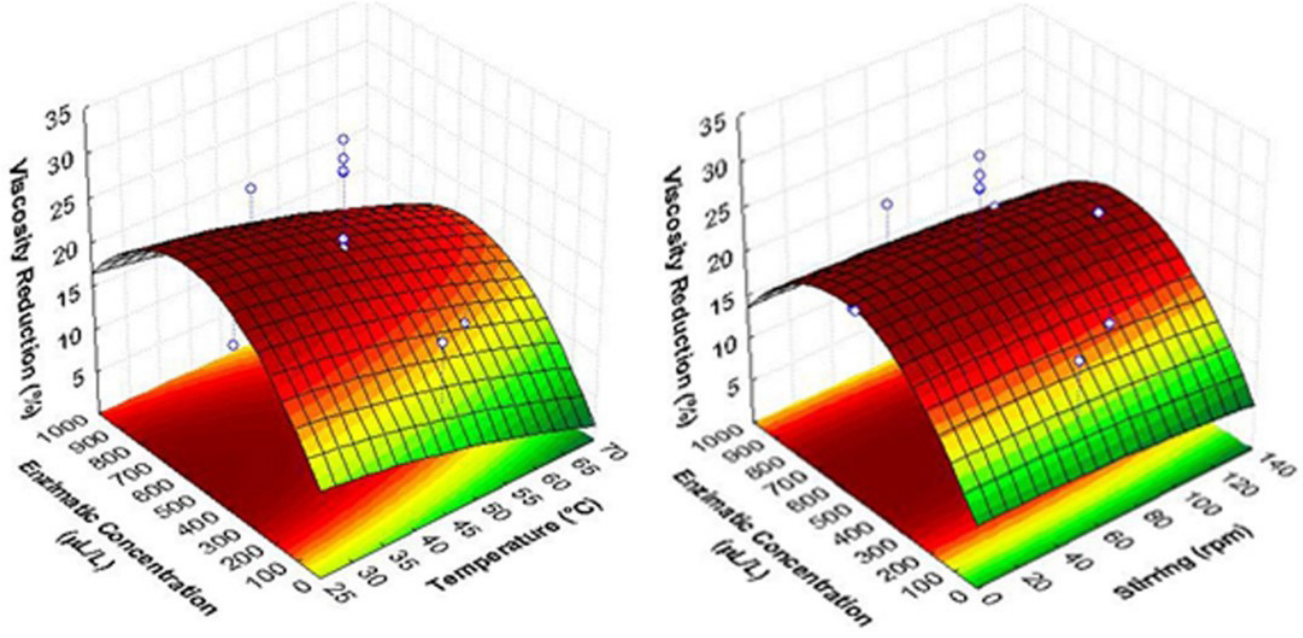

Figure 4. Response surface and response profile for viscosity reduction of free pectinase as a function of temperature and enzyme concentration and enzyme concentration and stirring.

with less than 40% of the initial activity. The loss of enzyme to the reaction medium was attributed to this reduction of activity.

Arya & Srivastava (2006) evaluated the activity of CGTase (cyclodextrin gluconotransferase) from *Bacillus macerans* ATCC 8244 encapsulated with calcium alginate for seven cycles. Seventy five per cent of the initial activity was maintained during the seven cycles. Wu et al. (2010) observed that the production of benzaldehyde by oxidation of benzyl alcohol by *Gluconobacter oxydans* M5/ALDH encapsulated with calcium alginate maintained 50% of its activity during 10 cycles.

3.3 Microfiltration of cagaita pulp

Cagaita pulp permeate flux

The mean flow of non hydrolyzed pulp after 2400 mL of MF was 68.8 L/m^2 · h, while hydrolyzed pulp was 78.0 L/m^2 · h resulting in a significant yield increase ($p \leq 0.05$) of 13.4%.

The non hydrolyzed pulp showed initial decline in the flux which became slightly ascending from 550 mL. The flow of the non hydrolyzed pulp remained slightly upward since the beginning of the process. The fouling phenomenon was not observed in any MF processes. The difference in behavior could be explained by the lower presence of pectic and cellulosic polymers in the hydrolyzed pulp which would lead to a longer time for pore blocking and the formation concentration polarization and/or interfacial gel layer profiles on the surface of the membrane.

A similar behavior to that presented in the hydrolyzed pulp was observed by Verma & Sarkar (2015) during the apple juice UF. The marked decrease in permeate flux observed at the UF beginning can be attributed to the pore blockage by pectic substances and increased concentration polarization. The slower flow, in later stages, can be attributed to the gel formation layer on the membrane surface.

In contrast to this study, Castro et al. (2007), studying the flux pattern of whole and hydrolyzed cashew juice clarified by MF (ceramic membrane with 0.1 mm pore and 0.005 m^2 area) and UF (30-80 kDa PVDF membrane and 0.05 m^2 area), observed higher mean fluxes (300 L/m^2.h) in the clarified MF juices compared to those clarified by UF (140 L/m^2.h).

On the other hand, no flux increase was related to the previous enzymatic hydrolysis in relation to the whole juice, since the hydrolyzed juice flux was smaller than that of the whole juice in the different membranes.

Maktouf et al. (2014) also obtained an increase in the permeate flux after the hydrolyzed lemon juice UF with the pectinase of *Penicillium occitanis* Pol6. The flux has been affected by the amount of high molecular weight pectic substances present therein.

Vaillant et al. (1999) evaluated the effect of 4 commercial pectinases on permeate flux in the passion fruit juice MF in a multi-channel ceramic tubular membrane. All enzymes treated had a positive effect on the permeate flux, but the magnitude of the effect was dependent of the commercial enzyme used. Enzymes containing mostly pectinase or cellulase had a reduced effect compared to those with substantial levels of both enzyme types.

Cianci et al. (2005), clarifying hydrolyzed cashew juice with a polyethersulfone tubular membrane (0.3 μm) at 220 kPa (2.2 bar), obtained a mean flux of 184 L/m^2 · h., equivalent to a 40% in relation to the flux using the non-hydrolyzed cashew juice.

Carvalho & Silva (2010) obtained mean fluxes of 57.55 and 46.85 L / m2 · h in hydrolyzed pineapple juice (MF), at 1.5 and 3.0 bar, respectively, using the same membrane type.

Bhattacharjee et al. (2007) evaluated the permeate flux of apple juice treated with 4 concentrations of the commercial Pectinex 3XL (pectinase) observing a 3-fold increase in the mean permeate juice flux during the UF.

Carvalho & Silva (2010) obtained higher yield in clarified hydrolyzed pineapple juice of 62.5% and 64.48% during MF at 1.5 and 3.0 bar, respectively.

Sreenath et al. (1994) obtained higher yields of pineapple juice previously hydrolyzed with commercial pectinase and cellulase (81-86%) compared to non-hydrolyzed juice (72%).

4 Conclusions

The optimal conditions of the cagaita pulp hydrolysis, considering the increase of clarity and the viscosity reduction, with free commercial enzyme, were: temperature of 38.9 °C, concentration of 483.1 μL/L and stirring of 93.9 rpm, and for the encapsulated: temperature of 30 °C, without stirring and enzymatic concentration of 570 μL/L. The conditions for the encapsulated enzyme were better than for the free enzyme, since they allowed to act at lower temperatures without stirring, which facilitates the process and the small increase in the enzyme amount was compensated by the possibility of reuse. After 8 cycles of use, the encapsulated enzyme maintained 30% of its activity in reducing the viscosity, resulting in the possibility of reuse, in contrast with the free enzyme that was lost just after the process. The mean flux during the MF of hydrolyzed cagaita pulp was 13.4% higher than that of non hydrolyzed one, indicating that the enzymatic treatment was efficient in reducing the process time.

The application of encapsulated commercial pectinolytic enzymes was satisfactory with the obtaintion of relevant data not, previously, reported for the fruit and applicable for the clarification of both cagaita pulp and other fruit pulps with similar physical and chemical characteristics. These encapsulated enzymes can be applied by the juices and fruit pulps industries as a step prior to MF and UF membranes processes increasing the permeated flux. In addition, the encapsulated enzymes and the possibility of reuse in up to 8 cycles will greatly reduce the operating costs of the juice processing industries.

Acknowledgements

The authors would like to thank CNPq, FAPERJ and Embrapa Food Technology and this work was supported by the CAPES (Coordenação de Aperfeiçoamento de Pessoal de Nível Superior - Brasil), CNPq and FAPERJ.

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