



## Effect of enzymatic treatment on the viscosity of raw juice and anthocyanins content in the microfiltrated blackberry juice

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### ABSTRACT

Fruit juices consumption has increased in recent years due to the perception of juices as a natural source of compounds that provide health benefits, such as anthocyanins, an important natural antioxidant. For the industrial production of blackberry, the juice microfiltration process is commonly used, but due to the high viscosity of this fruit, an enzymatic pretreatment is necessary. The objectives of this study were to select the operating variables (temperature and enzyme concentration) for the juice pretreatment and to evaluate its effect on the anthocyanins content in clarified blackberry juice. An experimental design was carried out to evaluate the rheological behavior of the juice as a function of temperature and enzyme concentration. The experimental data was analyzed by Ostwald-de-Waele model. The data of apparent viscosity as a temperature function was fitted by Arrhenius equation. The conditions of 35°C and 4 ml of enzyme Rapidase/kg of blackberry juice<sup>®</sup> TF from DSM Food Specialities were selected for the enzymatic liquefaction of cell-wall polysaccharides prior to microfiltration. Under this conditions it was possible to observe at the end of the process that this step leads to a considerable increase and maintenance of the permeate flux and an increase in the juice anthocyanin content.

*Keywords:* Cyanidin-3-O-glucoside; Cyaniding-3-O-rutenoside; Microfiltration; Rheological behavior

### 1. Introduction

The blackberry (*Rubus spp.*) culture has become a very attractive alternative when compared to other fruits due to the lower costs of deployment and maintenance of its orchard. Lower costs are due mainly to the fact that it is a rustic culture, with a lower incidence of pests and greater adaptation to different soil and climatic conditions [1].

The blackberry fruit (*Rubus spp.*) contains 85% of water, 10% of carbohydrates, besides being a natural source of functional compounds such as ellagic acid and anthocyanins [2,3]. Besides the function as pigments, anthocyanins have antioxidant activity, and due to this functional property they have been widely studied in recent years [4]. The large market for blackberry products is generated from the clarified and concentrate juice, which is the basis for preparation of a wide range of products such as syrups for ice cream, jellies, alcoholic and soft drinks [5].

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Interest in fruit juices has increased in the last fifteen years, with a great impact on Brazil economy, which is the largest producer and exporter in developing countries [6,7]. The application of membrane separation processes in the production of juices is a promising technique, since it has been indicated as a potential alternative to reduce the nutritional and sensory losses that may occur in the processes commonly used for conservation, clarification and concentration of these [8,9].

There are reports that in fruits like strawberries, cherries, blackberries and plums, the juice is retained within the cell structure and must be released. The blackberry usually has a large concentration of pectin polysaccharide, which makes its pulp highly viscous. Thus, for the use of membrane technologies, it becomes necessary to evaluate their rheological properties and propose viable alternatives to reduce its viscosity since this feature is directly linked to the flow properties of the pulp by the membrane system [10].

Enzymatic preparations, when added to fruit, promote breakdown of cell wall, hydrolyzing pectolytic compounds. Thus, the transport of juice is facilitated [5]. This feature can increase the yield of some substances in the fruit when subjected to the process of clarification.

Knowledge of the rheological parameters is important, not only in industrial applications to determine the energy consumption required to pump a viscous fruit pulp, but also to solve problems such as incorporation of air, which can cause difficulties in the operation of pumping and undesirable reactions as oxidation, and contamination [11].

The rheological behavior of fruit juices in general can not be described as a Newtonian fluid. For non-Newtonian fluids the relation between shear rate and shear stress is not constant. Several models have been used to characterize the flow behavior of fruit pulps, amongst which one of the most used is the Power Law also known as Ostwald-de-Waele model [12].

The objective of this study was to evaluate, by the technique of High Performance Liquid Chromatography (HPLC), the influence of enzymatic pretreatment and clarification by microfiltration on the concentration of blackberry majority anthocyanins, cyanidin-3-O glucoside and cyanidin-3-O-rutenoside.

## 2. Methodology

The blackberry used was stored at  $-18^{\circ}\text{C}$  for the period of two months, until the beginning of the microfiltration process. The fruit, collected at Rio Grande do Sul State, Brazil, was supplied by the company Mais Fruta Indústria e Comércio Ltda.

The blackberry juice was obtained by the depulping of the fruit in a depulper Itametal (Model Compacta/

Phase 220 M/ No 356/ CV 1), with a single stage, operating by gravity at room temperature.

The samples viscosity analyses were performed in concentric cylinders rheometer AR-G2, from TA Instruments®, coupled to the software Rheology Advantage Control AR. The temperature was set in the typical operation range ( $20\text{--}35^{\circ}\text{C}$ ) of fruit juices microfiltration process that takes into account the thermolabile characteristics of the antioxidant compounds.

The experiments were conducted in duplicate for the blackberry raw juice and for samples previously subjected to an enzymatic pretreatment. The enzymatic extract used was the Rapidase® TF from DSM Food Specialities. The enzymatic extract concentrations tested are presented in Table 1. That range of concentration was selected based on literature data for rich in pectin fruits microfiltration [13].

The influence of temperature on the apparent viscosity of non-Newtonian fluids was expressed in terms of an Arrhenius-type equation (Eq. (1)), involving the absolute temperature (T), the universal gas constant (R), the preexponential factor ( $\eta$ ) and the energy of activation for viscosity (Ea) [14]. The data were fitted using the linear regression method

$$\eta = \eta_0 e^{\frac{-Ea}{RT}} \quad (1)$$

where  $\eta$  is the apparent viscosity (cP);  $\eta_0$  is the apparent viscosity (cP) at  $T_0$ ; Ea is the activation energy (kJ/mol); R is the gas constant ( $8.314472 \text{ kJ}/(\text{kmol K})$ ); T is the temperature (K).

The rheological behavior of raw juice and hydrolyzed samples were analyzed by the Ostwald-de-Waele model (Eq. (2)).

$$\eta = K \times \left( \frac{dv}{dy} \right)^{n-1} \quad (2)$$

where K is the consistency index ( $\text{Pa}\cdot\text{s}^n$ ); n is the behavior index;  $\eta$  is the apparent viscosity (cP);  $dv/dy$  is the shear rate (1/s).

Table 1  
Experimental design for the viscosity analysis

Samples	Viscosity analysis			
Raw juice	20°C	25°C	30°C	35°C
Juice + 2 ml/kg of enzyme Rapidase*	20°C	25°C	30°C	35°C
Juice + 4 ml/kg of enzyme Rapidase*	20°C	25°C	30°C	35°C
Juice + 6 ml/kg of enzyme Rapidase*	20°C	25°C	30°C	35°C

\*Treatment for 30 min at  $35^{\circ}\text{C}$ .

The enzyme concentration effect on the juice viscosity was evaluated by Fisher test ( $p < 0.05$ ) using XLSTAT software (version 2006), from Addinsoft®.

The enzymatic treatment of blackberry raw juice was performed in the selected conditions with 400 ppm of Rapidase enzyme at 35°C for 30 min. After enzymatic treatment the juice was centrifuged in a basket centrifuge, SIZE 2 model, at 406 g. In order to clarify the blackberry juice, microfiltration was conducted at 35°C in a tubular membrane system composed of three  $\alpha$ -alumina membranes in series with pores diameter of 0.1 mm and total filtration area of 0.0165 m<sup>2</sup>.

The majority anthocyanins quantification was performed by High Performance Liquid Chromatography on a Waters® Alliance 2695 system, with a Waters® 2996 diode array detector using methodology described by Brito et al. [15], with adaptations by Araujo et al. [16]. The used column was a Symmetry® C18 (150 mm x 4.6; 3.5  $\mu$ m). The mobile phase consisted of 10% aqueous formic acid (solvent A) and methanol (solvent B). An external standard curve of each anthocyanin, cyaniding-3-O-glucoside and cyaniding-3-O-rutenoside, was made with analytical standards isolated from freeze-dried açai (*Euterpe oleraceae* Mart.). The isolation procedure was performed by HPLC, using an adapted six channels selecting valve connected after the detector, being used as collecting device, as described by Gouvêa et al. [17].

### 3. Results

The blackberry raw juice viscosity decrease severely after enzymatic pretreatment (Table 2). The Fisher's test ( $p < 0.05$ ), applied to mean comparison, suggest that blackberry juice viscosity values reached after pretreatment with 2 ml of enzyme/kg of blackberry juice was slightly superior as compared to pretreatment with 4 and 6 ml of enzyme/kg of blackberry juice. The pretreatment with 4 ml/kg was chosen to be used at the microfiltration process, because in this enzyme concentration was possible to obtain statistically the same reduction on the juice viscosity than the one obtained with 6 ml/kg, but with a lower consume of the enzymatic extract, wich makes the pretreatment more economically viable.

Table 2  
Results groupings classified, using XLSTAT software (version 2006)

Categories (ml/kg)	Mean (cP)	Groupings
0	80,000	A
2	5315	B
4	4260	C
6	4230	C

Table 3 shows the values of Power Law Model (Ostwald-de-Waelle) parameters obtained by fitting the experimental data for the blackberry juice treated with 4 ml of enzyme Rapidase/kg of blackberry juice at 20, 25, 30 and 35°C.

The behavior index ( $n$ ) was inferior to 1 for the enzymatically treated blackberry juice. The curve in Fig. 1 indicates a non-Newtonian behavior with pseudoplastic characteristics. As expected, the fluid consistency index ( $K$ ) was very sensitive to temperature.

Through linear fit of apparent viscosity values, calculated with shear rate set on the values of 10/s, 50/s, 100/s and 1000/s as a function of temperature inverse, the parameters of the Arrhenius equation were determined (Table 4). The determination coefficient ( $R^2$ )

Table 3  
Parameters of power law model of the depectinized blackberry juice as a function of temperature

Temperature	$n$	$K$ (Pa.s <sup><math>n</math></sup> )
20°C	0.28	169.88
25°C	0.33	90.80
30°C	0.35	84.52
35°C	0.36	67.53

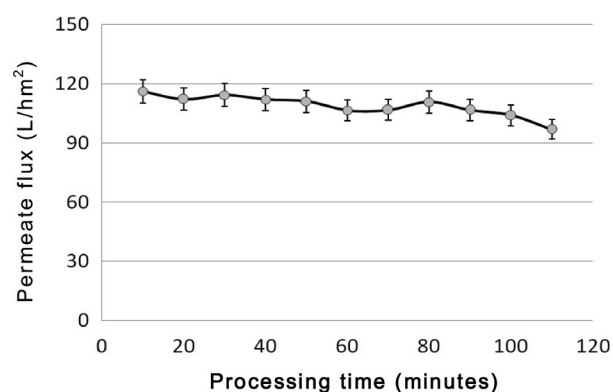


Fig. 1. Behavior of permeate flux in microfiltration processing of blackberry juice previously subjected to a treatment with 4 ml/kg of Rapidase enzymatic extract at 35°C.

Table 4  
Arrhenius equation parameters for the blackberry juice treated at 35°C with 4 ml/kg of Rapidase enzyme

Arrhenius parameters	Shear rate/s			
	10	50	100	1000
$E_a$ (kJ/mol)	28.84	26.08	24.60	10.28
$R^2$	0.91	0.92	0.93	0.96

obtained indicate that the equation represented a good fit to the apparent viscosity data for all shear rates selected. High values of activation energy ( $E_a$ ) suggest that the blackberry juice viscosity is relatively sensitive to temperature change.

The enzymatic pretreatment of blackberry raw juice promoted a significant reduction in the juice viscosity and a positive effect on the permeate flux during microfiltration step, where it was possible to observe the maintenance of a high value for it during all the process. In the classic profile of microfiltration, due the accumulation of material retained on the membrane surface, the flow reduces systematically during the process, a phenomenon known as fouling. In this study a significant reduction in the value of the permeate flux was not observed, which indicates that the enzyme treatment was effective in the hydrolysis of macromolecules responsible by fouling. This effect was also observed by Lukanin et al. [18], where in the concentration of apple juice by membrane, observed that the use of protease enzyme treatment increased the permeate flux. Authors attributed this improvement in the flow due to the reduction of biopolymers (including proteins) present in the juice after enzymatic hydrolysis. In fact, without the enzyme treatment, the permeate flux was so low that its determination was not feasible. Fig. 1 shows the behavior of permeate flux during the microfiltration process of blackberry juice after enzyme treatment. The flux value was similar to that reported by Vaillant et al. [8] for passion fruit juice after partial enzymatic liquefaction.

It can be observed an increase in anthocyanins concentration in the blackberry juice after enzymatic liquefaction, when compared to raw juice (Table 5). Consequently, the enzymatic treatment of blackberry has promoted an increase in the availability of two major anthocyanins. There was a 69% increase in the level of cyanidin-3-O-glucoside after pretreatment, while for cyanidin-3-O-rutenoside was observed an increase of 77.8%. Similar results were observed by [5], which registered an increase in the clarified blackberry juice anthocyanins extraction after the use of enzymes.

In the microfiltration process, 43.1% of cyanidin-3-O-glucoside present at feed stream passed into the

permeate, while 12% were retained by the membrane. Compared to cyanidin-3-O-rutenoside, 51.5% were permeated and 9.2% were retained. The mass balance showed a loss of 44.9% and 39.3% of the anthocyanin cyanidin-3-O-glucoside and cyanidin-3-O-rutenoside, respectively, during the process.

#### 4. Conclusion

Treatment with 4 ml of hydrolytic enzyme/kg of blackberry juice resulted in the blackberry juice viscosity reduction, and made possible the juice clarification process by microfiltration. It was also observed that the enzymatic treatment increased the concentration of anthocyanins in blackberry juice. The greater availability of anthocyanins in the juice after enzymatic liquefaction of cell wall provides the similar increase in other streams, mitigating the effects of losses associated with the processing. Moreover, the results observed in the juice clarification suggest the importance of improving the process conditions operations to avoid the anthocyanins losses and to allow that the blackberry clarified juice shows the similar quality of raw juice.

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Table 5  
Anthocyanins content in the raw and hydrolyzed blackberry juice streams

	Cyanidin-3-O-glucoside (mg/100 g)	Cyanidin-3-O-rutenoside (mg/100 g)
Raw juice	32.89	2.30
Hydrolyzed juice	55.61	4.09

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