Anais da Academia Brasileira de Ciências (2016) 88(1): 423-436
(Annals of the Brazilian Academy of Sciences)
Printed version ISSN 0001-3765 / Online version ISSN 1678-2690
http://dx.doi.org/10.1590/0001-3765201620140136
www.scielo.br/aabc

# Improvement on the concentrated grape juice physico-chemical characteristics by an enzymatic treatment and Membrane Separation Processes

**PLÍNIO R.F. CAMPOS[1], APARECIDO N. MÓDENES[2], FERNANDO R. ESPINOZA-QUIÑONES[2], DANIELA E.G. TRIGUEROS[2], SUELI T.D. BARROS[1] and NEHEMIAS C. PEREIRA[1]**

[1]Programa de Pós-Graduação em Engenharia Química, Universidade Estadual de Maringá/UEM, Av. Colombo, 5790, 87020-900 Maringá, PR, Brasil
[2]Programa de Pós-Graduação em Engenharia Química, Universidade Estadual do Oeste do Paraná/UNIOESTE, Rua da Faculdade, 645, Jd. La Salle, 85903-000 Toledo, PR, Brasil

*Manuscript received on March 31, 2014; accepted for publication on July 1, 2015*

**ABSTRACT**

In this work, the improvement on the concentrated grape juice physico-chemical characteristics by using an enzymatic treatment followed by Membrane Separation Process (MSP) has been investigated. By using Novozym 33095® and Ultrazym AFP L® enzymes varying three operating parameters, the best result on the grape pulp characteristics was attained for the Novozym 33095® performed at 35 $^{o}$C, 15 min. and 50 mgL$^{-1}$. In micro/ultra filtration processes after enzymatic pretreatment, the best performance of the MSP with high permeate flux value and suitable grape juice characteristics was attained using 0.05 μm membrane pore size, 1 bar pressure and 40 $^{o}$C treatment temperature. When reverse osmosis process is operated at 40 bar and 40 $^{o}$C, high soluble solid and low turbidity values are attained. An enzymatic treatment along with MSP has shown an alternative and efficient grape juice processing system, being possible to extend to other foods.

**Key words**: grape juice, enzymatic treatment, micro/ultra filtration, reverse osmosis.

## INTRODUCTION

Innovative technologies are required to meet quality standards and other demands of the consumer market as for production and marketing of different fruit juices. In order to attend the consumer's preference such as flavor, aroma, appearance and mouth feel, the juice industry has developed new techniques for retaining these characteristics of freshly squeezed juices in concentrate and in the reconstituted juice, but still distinguishable from fresh juice (Jiao et al. 2004).

Usually, fresh fruit juices consist largely of water (around 80%), with a high concentration of colloids that are rich on polysaccharides such as pectin, cellulose, hemicellulose, and lignin, among other substances (Vaillant et al. 2001). In particular, fresh grape juice presents an elevated acidity due to the presence of tartaric, malic and citric acids, ensuring a low pH value and equilibrium between acidic and sweet tastes (Gurak et al. 2010). Besides these characteristics, the fresh grape juice quality is also associated to a high amount of phenolic

Correspondence to: Fernando Rodolfo Espinoza-Quiñones
E-mail: f.espinoza@terra.com.br

compounds that are responsible by affecting colour and astringency (Girard and Mazza 1998). Due to be a differentiated beverage with positive energetic, nutritional and bioactive effects, grape juice was also reported as benefic to human health, reducing or preventing a wide range of diseases such as cancer (Jang et al. 1997, Thomasset et al. 2006, Gurak et al. 2010). Furthermore, technological advances on the phenolic compounds-enriched grape juice production as well as the impact of winemaking processes on phenolic extraction in wine have been reported (González-Barrio et al. 2009, Pérez-Lamela et al. 2007, Sacchi et al. 2005). Nonetheless, regarding its rich constitution, the cloudiness of the fresh fruit juice is mainly related to the presence of pectins, which are difficult to remove except by enzymatic treatment using pectinases (Vaillant et al. 1999, Kashyap et al. 2001). Many works on the optimization of enzymatic pretreatment for clarification of fruit juice have been reported in this regard (Lee et al. 2006, Rai et al. 2004, Sin et al. 2006). In addition, performing a pectin degradation, it is expected to reduce the membrane fouling, which is mainly caused by the colloidal constituents of the filtered media, resulting in a consequently drop on the flux in filtration processes (Balischi et al. 2002, Barros et al. 2003, Habert et al. 2006, Peter-Verbanets et al. 2011, Qu et al. 2012).

Many efforts have been devoted to improve methods such as freeze concentration, sublimation concentration and membranes for concentrated juice processing (Chen et al. 1993, Köseóglu et al. 1990). However, the Membrane Separation Process (MSP) including microfiltration, ultrafiltration and reverse osmosis is an advanced technique that has been widely applied to the dairy, food and beverage industry, allowing to clarify, concentrate, fractionate, desalt and purify fruit juice with low thermal damage to product, reduction in energy consumption and lower capital investments, because MSP is performed at low temperatures as well as it does not involve phase change for water removal (Jiao et al. 2004, Merson et al. 1980).

In last decades, traditional filtrations methods have been replaced by cross-flow microfiltration in oenology. After the firsts trials of cross-flow microfiltration with unsuitable results on the wine quality, the development of new filtration membrane along with a better understanding of the compounds involved in the membrane fouling have brought the selection of membrane suitable for wine filtration. In spite of progress made, some technological and economical barriers associated to the membrane fouling are still a limitation of the widespread application of this technique (El Rayess et al. 2011). Unfortunately, even though macromolecules present in clarified fruit juice are much smaller than the pore size of typical microfiltration membranes, they cause significant fouling (Czekaj et al. 2000).

The purpose of this paper is to study the improvement of the clarifying and concentrating grape juice quality concomitant with a minimization of the membrane fouling when a selected enzyme and better operating conditions are used in an integrated filtration system. Two different commercial enzymes (pectin lyase and polygalacturonase) were tested as a pretreatment step of the grape pulp in order to reduce the fouling phenomenon. Selection of the best enzyme along with finding better experimental condition were performed on the basis of the total acidity, soluble solids ($^{o}$Brix), color, total solids, and turbidity of the grape pulp as indicators of grape juice quality. For micro/ultra filtration processes, an inorganic tubular ceramic membrane operated under cross-flow mode was used to assess the influence of the processing parameters (pressure, temperature and pore size) on permeate flux and quality of clarified grape juice. By using a reverse osmosis unit, the improvement on the quality of concentrated grape juice was also assessed. In addition, the fouling mechanism was evaluated for designing more efficient filtration processes for the fruit industry.

## MATERIALS AND METHODS

### Collection and Preparation of the Pulp

In Brazil, the most common grape used to the manufacture of grape juice is the American species *Vitis labrusca*, commercially known as Isabel grape. A great amount of Isabel grape was purchased from a local harvest in the region of the city of Toledo, located in the Brazilian Paraná State. In lab, all grapes were washed, removed all type of particulates on them and submitted to a pulping by an industrial mechanical pulper. In addition, a mixture of grape pulps was performed and immediately distributed in several samples of 15 kg, packing in clean plastic bags and stored at -4 $^{o}$C for posterior analysis and treatment.

### Tested Enzymes

For a previous enzymatic treatment to MSP, both pectin lyase (Novozym 33095) and cellulase polygalacturonase (Ultrazym AFP L®) enzymes were tested. In order to assess the performance of both enzymes, concentration values of 50, 100 and 150 mgL$^{-1}$ were chosen, considering temperature values within the best pectolytic activity region (35 and 45 $^{o}$C for Novozym 33095 and 25 - 35 $^{o}$C for Ultrazym AFP L), according to the manufacturer manual (Novozymes Manual 2001).

### Physico-Chemical Analyses

As response physic-chemical parameters on the grape juice quality, the titratable acidity (TA), soluble solids, color, total solids, and turbidity were considered for non- and treated grape pulp samples as well as clarified and concentrated grape juice samples. For pH determination, a pH-meter (Digimed, model DM20) was used. Total solids were determined as AOAC (AOAC 1990). Color of grape juice samples coming from pulping and treatments was related to the light absorbance at a 440 nm wavelength and determined by using a spectrophotometer UV-vis (Shimadzu, model HACH DR/2010). By using the same spectrophotometer and setting the light absorbance at 860 nm, the turbidity of each grape juice sample was measured. A titrimetric method (AOAC 1990), consisting in titrating 10 mL sample with 0.1 N NaOH to pH 8.1 and using phenolphthalein as indicator, was applied to measure the TA as expressed in g tartaric acid per 100 mL of sample. Concentration of soluble solids (in $^{o}$Brix) was determined with a Shimadzu Abbe type refractometer Model 3L.

### Enzymatic Treatment

Based on a completely randomized design with 24 experiments in triplicates for each enzyme, a set of enzymatic experiments was performed for assessing the best experimental condition. According to the enzymatic treatment procedure, reported by Balischi et al. (2002), samples of 100 mL grape pulp placed in 250 mL-Erlenmeyer flasks were stirred in a thermostatic bath. Regarding two temperature for each enzyme, grape pulp samples were submitted to enzymatic treatment by adding concentrations of 50, 100 and 150 mg enzyme L$^{-1}$. Such treatments were performed at pectolytic activity temperatures (35 and 45 $^{o}$C for the Novozym 33095® and 25 and 35 $^{o}$C for the Ultrazym AFP L®) during adequate treatment times, ranging from 15 to 90 min at intervals of 15 min. After each enzymatic treatment, the thermostatic bath temperature was then increased to 70 $^{o}$C at least 20 minutes for the enzymatic deactivation. Control experiments without adding enzyme were also included. After performing all enzymatic treatments, aliquots of each treated sample were collected and analyzed, obtaining a set of response physic-chemical parameter data for each condition of treatment. In order to highlight the significant statistical difference between multiples mean values of response physic-chemical parameters, F-

and Tukey tests were applied to the experimental data and their results validated by ANOVA.

### MSP Treatments

Regarding the best enzymatic pretreatment experimental condition for the Novozym enzyme, a completely randomized design with several experiments was applied to all treatments of micro/ultrafiltration. In order to retain the particles of the grape juice after enzymatic treatment, a set of 18 micro/ultrafiltration experiments in triplicates were performed by using an ultrafiltration pilot unit (Netzsch, model 027.06-1C1/07-0005/AI). Such pilot unit consists of a feed tank of 5 L connected to a tubular module with 25 cm length and 0.7 cm internal diameter, being used ceramic membranes (α-Al2O3/TiO2). This MSP unit was operated under cross-flow mode with membranes of 0.05, 0.1 and 0.2 μm and 4.17 m/s maximum cross-flow velocity. By using a pumping, enzymatically pretreated sample was introduced to the MSP module that was operated at three pressures (1, 2 and 3 bar) and two temperatures (30 and 40 $^{o}$C), determining the permeate flux and analyzing the permeated grape juice for posterior treatment by reverse osmosis. Applying the Tukey test and ANOVA to the micro/ultrafiltration experimental data, significant statistical differences between multiples mean values of response physic-chemical parameters were highlighted.

Clarified grape juice samples, which were obtained from the micro/ultrafiltration unit under its better experimental condition, were considered to perform experiments based on the reverse osmosis process. A module of reverse osmosis consisting in a feed tank with a 15-L effective volume connected to a tubular module with 25 cm length and 0.7 cm internal diameter, being used composed film membranes of spiral type with 63.5 mm diameter and 355.6 mm length (FILMTEC, model BW30–2514). Samples from the feed tank were pumped into tubular module by a 4 HP motor, being the temperature and pressure of samples controlled. The reverse osmosis module was operated at a pressure of 40 bar and two temperatures (30 and 40 $^{o}$C), determining the permeate flux and collecting the concentrated grape juice for physic-chemical analysis.

### Determination of the Fouling Mechanism

By regarding the transient build-up of a yielded particulate layer of cake type on the membrane upstream interface in membrane processes, the permeate flux is negatively affected. Related to such a phenomenon, named as concentration polarization, the tendency is to occur a drastically reduction on the permeate flux at early filtration stage and drive gradually towards to a steady or nearly steady-state limit value after a long flux reduction. The physico-chemical interactions of the layer of rejected particulates with the membrane have been referred to another aspect of concentration polarization phenomenon. Another adversely phenomenon causing fouling at the interface is related to the adsorption on the membrane pore walls and pore plugging by solute penetrate (Barros et al. 2003). The membrane fouling is considered the main disadvantage of MPS application, due to the frequent cleanings or replacements of membranes and especially by increased operating costs caused by the higher power consumption, caused by the reduced permeate flux along time (Giorno et al. 1998).

Regarding constant pressure blocking filtration laws, earlier proposed by Hérmia (Hérmia 1982) who applied to power-law non-Newtonian fluids, and reformulated by Field et al. (1995) for cross-flow microfiltration in critical flux, the decay on the permeate flux in cross-flow filtration at constant pressure was proposed by Barros et al. (2003), being mathematical expressed by the Eq. 1. Attributing a suitable value for the general index (n) in Eq. 1 a kind of fouling mechanism involved during the filtration process could be evidenced (Barros et al. 2003). A complete blocking of membrane pores is

expected to n value equal 2. Beside this, an internal blocking of pores might occur as n assumes a value of 1.5. When n value is equal to 1, a partial blocking of pores is expected, while for n value equal to 0 a cake type layer is formed.

$$\frac{dJ}{dt} = -k\left(J - J^{*}\right) J^{2-n} \quad (1)$$

where J is the permeate flux, J* is the critical flux and t is the time. As reported by Todisco et al. (1996), k and n are phenomenological coefficient and general index, respectively, both depending on fouling mechanism.

A stochastically optimized global method, called a Particle Swarm Optimization (PSO), was applied to search the best modeling parameters (k and n) through a non-linear fitting of the experimental data. The basic principle of the PSO method is to seek a set of potential solutions located in a wide search hyperspace that is randomly scanned under different kinematic conditions of bird flocking according to some considerations based on local ($c_1$) and global ($c_2$) accelerations and swarm inertia (ω). An insight into these better values was reported earlier in other works (Espinoza-Quiñones et al. 2009, Trigueros et al. 2010a, b). Best results of attaining the near-global solution have been reported when both global and local collective accelerations have assumed the same value and are equal to 1.5 (Módenes et al. 2012, Trigueros et al. 2012). In this work, an initial particle swarm (at least 500) is defined as well as the number of iterations (at least 25) in order to scan a wide search hyperspace where potential solutions are identified and stored. The performance of each particle is related to a built-in objective function (OF), determined by the least square statistical method, as shown in Eq. 2 of the present work. Besides these parameters, the critical flux (J*) was included as a parameter to be determined during the search procedure. PSO method was implemented in the software Maple 14®, and executed within a Windows 7 environment, using a microcomputer Intel® CoreTM i7-930, 2.8 GHz and 8 GB RAM.

$$OF = \sum_{i=1}^{n} \left( \frac{J_i^{\exp} - J_i^{pred}}{J_i^{\exp}} \right)^2 \quad (2)$$

where $J^{exp}$ is the flux value obtained experimentally and $J^{pred}$ is the flux value predicted by the model.

## RESULTS AND DISCUSSION

### Grape Pulp Characterization

Analyzing the characteristics of the grape pulp sample in nature, values of 13.9 g tartaric acid per 100 mL of grape juice, 11.0 °Brix, 31,000 mg Pt-Co $L^{-1}$, 3.1, 7.7 and 8,700 FAU for titratable acidity, soluble solids, color, pH, total solids and turbidity, respectively, were found. The Brazilian standard for the grape juice quality is not well defined because it depends on achieving a set of specific characteristics such as flavor, taste, and appearance for final acceptance by the consumer. In general, these characteristics are mainly related to the grape origin, applied treatment systems to produce grape juice among factors, being possible to manufacture a great variety of grape juice.

In this work, an improvement on the grape juice quality by applying an enzymatic pretreatment followed by two Membrane Separation Processes was monitored by titratable acidity, total solids, soluble solids, color, pH and turbidity. However, some initial characteristics, such as pH of grape pulp samples in nature, are advisable to be maintained unchangeable. Meanwhile other characteristics, for instance, high concentrations of total solids and turbidity, are recommendable to reduce their values to lower ones after a series of grape pulp treatments, by applying more effective and low-cost processes, searching a grape juice of high quality along with a high acceptance by the consumer.

ENZYMATIC PRETREATMENT ANALYSIS

The most effective enzyme and the best experimental conditions for the enzymatic treatment were searched on the analysis of the physico-chemical parameter data obtained at three enzyme concentrations, and two pectolytic activity temperatures for each enzyme, regarding treatment time range from 0 to 90 min. From a completely randomized experimental design, the set of results for the titratable acidity, soluble solids (°Brix), color, pH, total solids and turbidity is obtained and summarized in Tables I and II as Novozym 33095 and Ultrazym AFP L enzymes, respectively, were used.

**TABLE I**
**Physico-chemical parameters of the grape pulp after treatment with the Novozym 33095 enzyme.**

| Parameter | pectolytic activity temperature (°C) | Enzyme Concentration (mg $L^{-1}$) | Enzymatic treatment time (min) | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | | 15 | 30 | 45 | 60 | 75 | 90 |
| Titratable acidity (g tartaric acid/100 mL sample) | 35 | 50 | 12.8±0.6 | 12.3±0.6 | 12.3±0.6 | 12.3±0.6 | 12.8±0.6 | 13.4±0.7 |
| | | 100 | 12.8±0.6 | 14.5±0.7 | 12.3±0.6 | 12.3±0.6 | 12.8±0.6 | 12.8±0.6 |
| | | 150 | 12.8±0.6 | 16.2±0.9 | 15.1±0.9 | 15.6±0.9 | 17.3±0.9 | 15.6±0.9 |
| | 45 | 50 | 13.9±0.7 | 13.4±0.7 | 12.8±0.6 | 13.4±0.7 | 13.4±0.7 | 13.9±0.7 |
| | | 100 | 12.8±0.6 | 13.4±0.7 | 15.1±0.8 | 16.2±0.8 | 13.9±0.7 | 14.5±0.7 |
| | | 150 | 16.7±0.9 | 17.3±0.9 | 16.7±0.9 | 17.3±0.9 | 16.7±0.9 | 17.3±0.9 |
| Soluble solids (oBrix) | 35 | 50 | 7.4±0.4 | 7.0±0.4 | 7.0±0.4 | 7.0±0.4 | 7.8±0.4 | 7.2±0.4 |
| | | 100 | 6.8±0.3 | 7.0±0.4 | 7.4±0.4 | 7.4±0.4 | 7.8±0.4 | 7.4±0.3 |
| | | 150 | 9.8±0.5 | 9.8±0.5 | 9.8±0.5 | 9.8±0.5 | 10.8±0.5 | 9.8±0.5 |
| | 45 | 50 | 11.0±0.6 | 13.9±0.7 | 10.9±0.6 | 10.9±0.6 | 13.1±0.7 | 11.9±0.6 |
| | | 100 | 12.9±0.6 | 13.9±0.7 | 14.5±0.7 | 13.8±0.7 | 12.1±0.6 | 11.1±0.6 |
| | | 150 | 12.0±0.6 | 11.2±0.6 | 11.2±0.6 | 11.8±0.6 | 11.8±0.6 | 11.2±0.6 |
| Color (1.000 mg Pt-Co/L) | 35 | 50 | 25.9±0.8 | 25.0±0.8 | 28.7±0.9 | 26.8±0.8 | 34.3±1 | 21.2±0.6 |
| | | 100 | 22.3±0.7 | 23.8±0.7 | 25.0±0.8 | 26.5±0.8 | 26.5±0.8 | 28.6±0.9 |
| | | 150 | 25.4±0.8 | 35.6±1.1 | 38.6±1.2 | 32.3±1.2 | 26.5±0.8 | 22.6±0.7 |
| | 45 | 50 | 22.3±0.7 | 25.6±0.8 | 27.0±0.8 | 29.7±0.9 | 36.5±1.1 | 28.6±0.9 |
| | | 100 | 29.2±0.9 | 43.7±1.3 | 26.8±0.8 | 38.7±1.2 | 36.3±1.1 | 35.7±1.1 |
| | | 150 | 20.1±0.6 | 26.0±0.8 | 23.1±0.7 | 19.8±0.6 | 21.3±0.6 | 29.4±0.9 |
| pH | 35 | 50 | 3.40±0.02 | 3.40±0.02 | 3.28±0.02 | 3.28±0.02 | 3.35±0.02 | 3.34±0.02 |
| | | 100 | 3.23±0.02 | 3.23±0.02 | 3.28±0.05 | 3.32±0.02 | 3.21±0.02 | 3.31±0.02 |
| | | 150 | 3.20±0.02 | 3.20±0.01 | 3.18±0.02 | 3.12±0.02 | 3.20±0.02 | 3.20±0.02 |
| | 45 | 50 | 3.29±0.02 | 3.26±0.02 | 3.19±0.02 | 3.18±0.02 | 3.15±0.02 | 3.08±0.02 |
| | | 100 | 3.23±0.02 | 3.23±0.02 | 3.23±0.02 | 3.22±0.02 | 3.27±0.02 | 3.21±0.02 |
| | | 150 | 3.10±0.02 | 3.15±0.02 | 3.16±0 | 3.07±0.02 | 3.10±0.02 | 3.15±0.02 |
| Total solids (%m/m) | 35 | 50 | 6.7±0.3 | 6.9±0.3 | 6.9±0.3 | 7.0±0.4 | 7.0±0.4 | 7.0±0.4 |
| | | 100 | 7.4±0.4 | 7.4±0.4 | 6.6±0.3 | 6.8±0.3 | 6.8±0.3 | 6.7±0.3 |
| | | 150 | 8.2±0.4 | 8.5±0.4 | 8.7±0.4 | 8.2±0.4 | 8.8±0.4 | 9.1±0.5 |
| | 45 | 50 | 7.4±0.4 | 7.4±0.4 | 7.3±0.4 | 7.4±0.4 | 6.9±0.3 | 7.7±0.4 |
| | | 100 | 7.4±0.4 | 8.9±0.4 | 8.6±0.4 | 8.9±0.4 | 9.0±0.5 | 8.5±0.4 |
| | | 150 | 9.5±0.5 | 9.6±0.5 | 9.4±0.5 | 9.4±0.5 | 9.4±0.5 | 9.2±0.5 |
| Turbidity (1.000 FAU) | 35 | 50 | 4.5±0.1 | 4.5±0.1 | 9.5±0.3 | 6.9±0.2 | 5.1±0.2 | 6.5±0.2 |
| | | 100 | 5.3±0.1 | 9.1±0.3 | 9.1±0.3 | 8.0±0.2 | 6.3±0.2 | 5.9±0.2 |
| | | 150 | 7.8±0.2 | 7.8±0.2 | 13.2±0.4 | 10.2±0.3 | 11.4±0.3 | 3.7±0.1 |
| | 45 | 50 | 6.5±0.2 | 7.3±0.2 | 8.3±0.3 | 7.3±0.2 | 9.3±0.3 | 10.9±0.3 |
| | | 100 | 5.5±0.2 | 6.3±0.2 | 11.4±0.3 | 11.1±0.3 | 12.3±0.4 | 12.5±0.4 |
| | | 150 | 4.2±0.1 | 6.3±0.2 | 5.0±0.2 | 4.0±0.1 | 6.1±0.2 | 7.5±0.2 |

**TABLE II**
**Physico-chemical parameters of the grape pulp after treatment with the Ultrazym AFP L enzyme.**

| Parameter | pectolytic activity temperature (°C) | Enzyme Concentration (mg $L^{-1}$) | Enzymatic treatment time (min) | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | | 15 | 30 | 45 | 60 | 75 | 90 |
| Titratable acidity (g tartaric acid/100 mL sample) | 25 | 50 | 17.3±0.9 | 17.3±0.9 | 17.3±0.9 | 16.7±0.8 | 16.2±0.8 | 15.1±0.8 |
| | | 100 | 14.5±0.7 | 15.6±0.7 | 17.3±0.8 | 16.7±0.8 | 17.3±0.7 | 17.9±0.8 |
| | | 150 | 15.6±0.9 | 15.1±0.9 | 14.5±0.9 | 14.5±0.9 | 14.5±0.9 | 15.6±0.9 |
| | 35 | 50 | 17.9±0.9 | 18.4±0.9 | 17.3±0.9 | 16.7±0.8 | 18.4±0.9 | 16.2±0.8 |
| | | 100 | 17.9±0.9 | 18.4±0.9 | 17.9±0.9 | 17.9±0.9 | 17.9±0.9 | 18.4±0.9 |
| | | 150 | 16.7±0.9 | 17.9±0.9 | 17.9±0.9 | 16.2±0.9 | 12.8±0.9 | 13.4±0.9 |
| Soluble solids (°Brix) | 25 | 50 | 11.1±0.6 | 11.1±0.6 | 11.0±0.6 | 11.1±0.6 | 10.2±0.5 | 9.7±0.5 |
| | | 100 | 9.2±0.5 | 9.9±0.5 | 10.2±0.5 | 10.9±0.5 | 11.0±0.6 | 11.0±0.6 |
| | | 150 | 10.2±0.5 | 10.2±0.5 | 10.0±0.5 | 10.6±0.5 | 10.4±0.5 | 10.4±0.5 |
| | 35 | 50 | 10.0±0.5 | 11.4±0.6 | 10.7±0.5 | 10.4±0.5 | 10.8±0.5 | 10.4±0.5 |
| | | 100 | 11.9±0.6 | 11.8±0.6 | 11.8±0.6 | 12.0±0.6 | 12.0±0.6 | 12.1±0.6 |
| | | 150 | 12.0±0.6 | 12.0±0.6 | 12.2±0.6 | 9.4±0.5 | 7.8±0.4 | 9.2±0.4 |
| Color (1.000 mg Pt-Co/L) | 25 | 50 | 33.3±1 | 37.0±1.1 | 38.9±1.2 | 35.7±1.1 | 42.1±1.3 | 31.1±0.9 |
| | | 100 | 33.8±1 | 33.0±1 | 39.3±1.2 | 32.2±1 | 37.4±1.1 | 39.2±1.2 |
| | | 150 | 37.6±1.3 | 35.5±1.1 | 33.5±1 | 34.7±1 | 32.2±1 | 31.2±0.9 |
| | 35 | 50 | 40.6±1.2 | 58.2±1.7 | 48.8±1.5 | 40.4±1.2 | 50.8±1.5 | 40.2±1.2 |
| | | 100 | 39.4±1.2 | 39.2±1.2 | 29.3±0.9 | 26.6±0.8 | 26.6±0.8 | 30.3±0.9 |
| | | 150 | 33.2±1 | 43.1±1.3 | 44.1±1.3 | 39.6±1.2 | 38.5±1.2 | 31.3±0.9 |
| pH | 25 | 50 | 3.21±0.02 | 3.16±0.02 | 3.16±0.02 | 3.19±0.02 | 3.17±0.02 | 3.16±0.02 |
| | | 100 | 3.20±0.02 | 3.16±0.02 | 3.15±0.02 | 3.05±0.02 | 3.00±0.02 | 2.91±0.02 |
| | | 150 | 3.07±0.02 | 3.15±0.02 | 3.11±0.02 | 3.09±0.02 | 2.98±0.02 | 2.86±0.02 |
| | 35 | 50 | 3.16±0.02 | 3.13±0.02 | 3.12±0.02 | 3.10±0.02 | 3.04±0.02 | 2.98±0.02 |
| | | 100 | 3.08±0.02 | 3.07±0.02 | 3.05±0.02 | 3.06±0.02 | 3.00±0.02 | 2.95±0.02 |
| | | 150 | 3.11±0.02 | 3.08±0.02 | 3.09±0.02 | 3.08±0.02 | 3.08±0.02 | 3.08±0.02 |
| Total solids (%m/m) | 25 | 50 | 8.9±0.4 | 8.9±0.4 | 9.1±0.5 | 8.2±0.4 | 8.0±0.4 | 8.0±0.4 |
| | | 100 | 6.6±0.3 | 8.0±0.4 | 8.7±0.4 | 9.2±0.5 | 9.4±0.5 | 9.2±0.5 |
| | | 150 | 9.7±0.5 | 9.6±0.5 | 9.4±0.5 | 8.9±0.4 | 9.3±0.5 | 9.3±0.5 |
| | 35 | 50 | 9.4±0.5 | 11.4±0.6 | 10.2±0.5 | 10.2±0.5 | 10.6±0.5 | 10.3±0.5 |
| | | 100 | 10.4±0.5 | 10.5±0.5 | 11.0±0.6 | 10.6±0.5 | 10.8±0.5 | 11.7±0.6 |
| | | 150 | 11.3±0.6 | 11.4±0.6 | 8.9±0.4 | 7.2±0.4 | 7.7±0.4 | 11.5±0.6 |
| Turbidity (1.000 FAU) | 25 | 50 | 7.7±0.2 | 8.0±0.2 | 8.8±0.3 | 9.1±0.3 | 14.2±0.4 | 9.0±0.3 |
| | | 100 | 10.4±0.3 | 7.8±0.2 | 10.0±0.3 | 7.4±0.2 | 12.1±0.4 | 8.7±0.3 |
| | | 150 | 14.0±0.4 | 12.3±0.4 | 8.1±0.2 | 5.0±0.2 | 5.4±0.2 | 3.1±0.1 |
| | 35 | 50 | 12.5±0.4 | 12.9±0.4 | 12.6±0.4 | 8.8±0.3 | 10.5±0.3 | 9.5±0.3 |
| | | 100 | 6.5±0.2 | 9.3±0.3 | 5.3±0.2 | 6.5±0.2 | 6.5±0.2 | 7.1±0.2 |
| | | 150 | 9.1±0.3 | 8.8±0.3 | 7.2±0.2 | 9.6±0.3 | 9.8±0.3 | 8.2±0.2 |

According to the null hypothesis test, all response physico-chemical parameter (titratable acidity, total solids, soluble solids, color, pH and turbidity) data have followed normal distributions. In addition, the application of F- and Tukey tests on the experimental data have been performed, showing very similar results (data not shown) related to the comparison between multiple mean values of response parameters (RP). For this reason, results of the Tukey test are only being reported in the present work.

The Tukey test was ran within the software SAS®, version 9.1, introducing as criterion of comparison among all multiple mean values of RP within a 95% confidence level. All Tukey results were validated by ANOVA (data not shown), providing the least mean value for each RP as well as allowing to highlight the best experimental condition for the set of enzymatic treatments.

Performing the Tukey test analysis of RP data obtained for the two tested enzyme types at the two temperatures for better pectolic activity, three enzyme concentrations, and seven treatment times, the lowest RP values were attained as Novozym 33095 enzyme is used at 35 ºC temperature, 15 min. treatment time, and 50 mgL$^{-1}$ concentration. Under the best experimental condition, the enzymatically treated grape juice was characterized by the lowest values of 12.82 g tartaric acid per 100 mL of grape juice, 11.0 ºBrix, 31,550 mg Pt-Co L$^{-1}$, 3.1 and 8,700 FAU for titratable acidity, soluble solids, color, pH and turbidity, respectively. A set of enzymatically treated grape juice samples was posterior submitted to experimental designs for assessing micro, ultra filtration and reverse osmosis processes.

## Membrane Separation Process Analysis

Regarding the set of treatments of enzymatically treated grape juice samples based on micro and ultra filtration processes within a completely randomized experimental design, a set of mean values of permeate flux (in kg m$^{-2}$ h$^{-1}$) and response physico-chemical parameters (RP) was obtained, as summarized in Table III. The filtration system unit was operated at three pressures (1, 2 and 3 bar), two temperatures (30 and 40 ºC) and by using three tubular ceramic membranes with pore diameter of 0.2 and 0.1 μm, for micro filtration, and 0.05 μm, for ultra filtration. It can be noticed that an increasing on the pore size from 0.05 to 0.2 μm has resulted in a decreasing permeate flux for both considered temperatures. A change on the temperature of the treated grape juice at high pressure has driven to a strong decay on the permeate flux when the ultra filtration system was operated with lower membrane pore size. Besides this, an improvement on the permeate flux value was achieved when low pressure and small pore size were used. It could be explained by the polarization effect present in the cake layer formation.

**TABLE III**

**Mean values of the permeate flux and physico-chemical parameters after micro and ultra filtration of enzymatically treated grape juice samples, which were obtained for the Novozym 33095 enzyme (50 mg L$^{-1}$, 35 ºC pectolyc activity temperature, and 15 min. pretreatment time).**

| Parameter | Temperature (ºC) | Pressure (bar) | Membrane pore diameter (mm) | | |
|---|---|---|---|---|---|
| | | | 0.05 | 0.1 | 0.2 |
| Mean permeate flux (kg m$^{-2}$ h$^{-1}$) | 30 | 1 | 99.54 | 76.91 | 83.01 |
| | | 2 | 96.54 | 77.83 | 63.01 |
| | | 3 | 100.44 | 118.00 | 79.28 |
| | 40 | 1 | 136.38 | 87.95 | 74.53 |
| | | 2 | 120.00 | 112.50 | 98.92 |
| | | 3 | 79.21 | 50.82 | 53.56 |
| Titratable acidity (g tartaric acid/100 mL sample) | 30 | 1 | 13.3±0.7 | 13.3±0.6 | 14.0±0.7 |
| | | 2 | 12.8±0.6 | 13.0±0.7 | 12.3±0.6 |
| | | 3 | 11.4±0.6 | 9.9±0.6 | 10.2±0.6 |
| | 40 | 1 | 12.1±0.6 | 12.4±0.6 | 13.9±0.7 |
| | | 2 | 10.4±0.6 | 9.8±0.6 | 11.4±0.6 |
| | | 3 | 10.4±0.6 | 14.5±0.7 | 12.8±0.6 |

**TABLE III (continuation)**

| Parameter | Temperature (°C) | Pressure (bar) | Membrane pore diameter (mm) | | |
|---|---|---|---|---|---|
| | | | 0.05 | 0.1 | 0.2 |
| Soluble solids (°Brix) | 30 | 1 | 10.9±0.6 | 10.6±0.6 | 10.8±0.6 |
| | | 2 | 9.8±0.6 | 10.4±0.6 | 11.0±0.6 |
| | | 3 | 8.0±0.6 | 8.4±0.6 | 8.5±0.6 |
| | 40 | 1 | 8.8±0.6 | 9.2±0.6 | 11.3±0.6 |
| | | 2 | 9.6±0.6 | 7.5±0.6 | 9.4±0.6 |
| | | 3 | 9.6±0.6 | 12.3±0.6 | 11.0±0.6 |
| Color (1000 mg Pt-Co/L) | 30 | 1 | 3.8±0.2 | 5.2±0.2 | 4.3±0.2 |
| | | 2 | 4.2±0.2 | 5.1±0.2 | 4.3±0.2 |
| | | 3 | 3.7±0.2 | 4.2±0.2 | 4.1±0.2 |
| | 40 | 1 | 3.0±0.2 | 4.3±0.2 | 4.1±0.2 |
| | | 2 | 3.5±0.3 | 2.7±0.2 | 5.5±0.4 |
| | | 3 | 3.5±0.2 | 6.1±0.5 | 5.5±0.4 |
| pH | 30 | 1 | 3.00±0.02 | 3.05±0.02 | 2.97±0.02 |
| | | 2 | 3.16±0.02 | 3.12±0.02 | 3.23±0.02 |
| | | 3 | 3.17±0.02 | 3.42±0.02 | 2.98±0.02 |
| | 40 | 1 | 3.03±0.02 | 3.46±0.02 | 2.94±0.02 |
| | | 2 | 2.85±0.02 | 3.58±0.02 | 3.45±0.03 |
| | | 3 | 3.20±0.04 | 3.01±0.02 | 2.89±0.02 |
| Turbidity (FAU) | 30 | 1 | 100±10 | 100±10 | 100±10 |
| | | 2 | 150±30 | 100±10 | 200±20 |
| | | 3 | 100±10 | 100±10 | 200±40 |
| | 40 | 1 | 150±30 | 100±10 | 200±20 |
| | | 2 | 150±30 | 200±20 | 150±30 |
| | | 3 | 150±30 | 150±30 | 150±30 |
| Specific mass (g $cm^{-3}$) | 30 | 1 | 1.04±0.01 | 1.04±0.01 | 1.04±0.01 |
| | | 2 | 1.03±0.01 | 1.04±0.01 | 1.04±0.01 |
| | | 3 | 1.03±0.01 | 1.02±0.02 | 1.03±0.01 |
| | 40 | 1 | 1.03±0.02 | 1.03±0.01 | 1.03±0.02 |
| | | 2 | 1.04±0.01 | 1.03±0.02 | 1.03±0.02 |
| | | 3 | 1.03±0.01 | 1.04±0.01 | 1.04±0.01 |

By analyzing the behavior of the permeate flux with the mean pore size and applied pressure, a reduction on the permeate flux was observed with an increasing on both parameter pore size and pressure values (see Table III). Experimental permeate flux data obtained from a micro and ultra filtration unit were fit by the model described by Eq. 1. The PSO method was applied in order to search the globally optimized values for the phenomenological coefficient (k) and the general index (n). All PSO results are shown in Table IV, while the behavior of the permeate flux values as function of time in ultrafiltration process, performed at 40 °C, 0.05 μm pore size and three pressures (1, 2 and 3bar), is shown in Fig. 1.

As a function of the solid/solute size and shape in relation to the membrane pore size, several types of modes are expected to occur, according to the n-value. According to Field et al. (1995) the critical flux corresponds to the permeate flux before the fouling, i.e., it consists on the highest permeate flux for which no flux reduction in time is observed. Regarding a temperature of 30 °C in processes of micro and ultra filtration, it can be

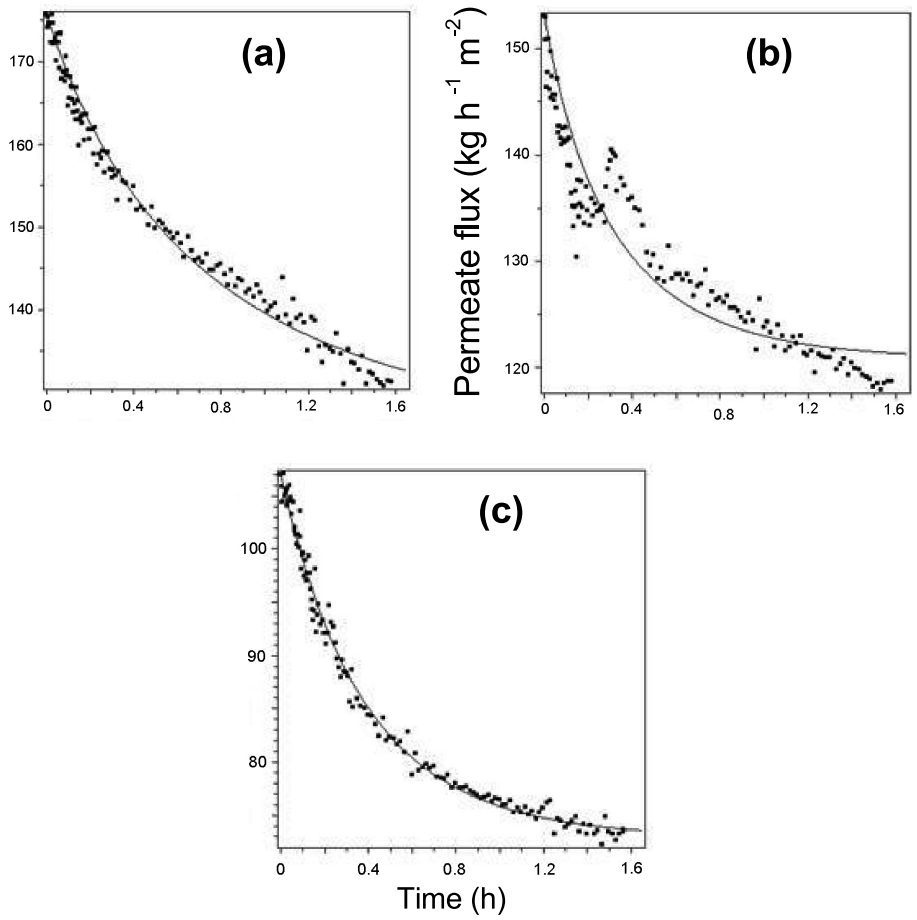


**Figure 1 -** Behavior of the permeate flux values as function of time in ultrafiltration processes performed at 40 °C, 0.05 µm pore size and pressures of **a**) 1, **b**) 2 and **c**) 3 bar, along with the respective fits of the tested model, proposed by Field et al. (1995).

**TABLE IV**
**Model parameters estimated by the PSO method for the experimental data in micro and ultra filtration processes.**

| Pore size (mm) | Temperature (°C) | Pressure (bar) | n | k | $J^{critical}$ | OF |
|---|---|---|---|---|---|---|
| 0.05 | 30 | 1 | 0 | 0.000052 | 124.514670 | 0.019271 |
| | | 2 | 0 | 0.000083 | 117.213004 | 0.030370 |
| | | 3 | 0 | 0.000127 | 113.544708 | 0.075947 |
| | 40 | 1 | 0 | 0.000052 | 124.514670 | 0.019271 |
| | | 2 | 0 | 0.000157 | 120.764639 | 0.079492 |
| | | 3 | 1.5 | 0.266188 | 72.912129 | 0.019308 |
| 0.1 | 30 | 1 | 0 | 0.000174 | 111.320648 | 0.032904 |
| | | 2 | 0 | 0.000130 | 109.825172 | 0.027529 |
| | | 3 | 0 | 0.000178 | 100.993001 | 0.020634 |
| | 40 | 1 | 0 | 0.000158 | 83.647887 | 0.027311 |
| | | 2 | 2 | 1.578215 | 106.919653 | 0.093469 |
| | | 3 | 2 | 2.178883 | 48.552319 | 0.043710 |

**TABLE IV (continuation)**

| Pore size (mm) | Temperature (°C) | Pressure (bar) | n | k | $J^{critical}$ | OF |
|---|---|---|---|---|---|---|
| 0.2 | 30 | 1 | 0 | 0.000521 | 93.349341 | 0.042743 |
| | | 2 | 0 | 0.000291 | 102.133143 | 0.079376 |
| | | 3 | 0 | 0.000157 | 85.523997 | 0.053352 |
| | 40 | 1 | 0 | 0.000611 | 76.274423 | 0.152547 |
| | | 2 | 2 | 4.310847 | 102.337374 | 0.224132 |
| | | 3 | 2 | 5.663746 | 51.907259 | 0.339970 |

noticed that the same mode of fouling associated to a cake layer formation (n=0) is expected to be present, regardless of tested membrane pore size and pressure values. However, considering a temperature of 40 °C, it was observed a systematic progress in the fouling mechanism mode, appearing first a cake layer formation (n=0) for low pressure value (1 bar) and ending with a complete blocking of membrane pores (n=2) for high pressure value (3 bar). In addition, as a consequence of an increasing on the pore size an increasing on the k value and a reduction of the critical flux (J*) value are expected. A similar response is expected to occur when an increasing on the temperature is considered, except to pore size of 0.05 μm for which the results for permeate flux were kept unaltered. The pressure effect on the complete blocking of membrane pores become more evident when greater membrane pore sizes are used. Although the permeate flux is positively favored as a consequence of a reduction of the viscosity at high temperatures, a complete blocking of pores could occur when the pressure is increased.

Relatively scarce information on detailed studies of fouling mechanisms caused by polysaccharides and polyphenols is found in literature (Czekaj et al. 2000). In earlier works (Belleville et al. 1990, 1992), performing microfiltration of red wine, membrane fouling has been attributed to high levels of polysaccharides and polyphenols. In ultrafiltration processes the interaction between inorganic particles and biopolymers has resulted in a fouling cake of significantly reduced porosity (Jermann et al. 2008). An irreversible fouling by organic matter takes place due to internal pore adsorption, affecting negatively the ultrafiltration process (Katsoufidou et al. 2005). In the case of the use of enzymatically treated grape juice samples, the pectin component might also agglomerate other particulates forming a fouling cake. Furthermore, there is a greater contribution in the transversal flux with an increasing on the pressure, reducing thus the permeate flux. As a consequence of an increasing on the temperature, the permeate flux is positively favored due to a reduction on the viscosity value of the grape juice, allowing carrying on particulates with high feasibility through a less viscous medium.

A statistical analysis of micro and ultra filtration processes was performed by the Tukey test, aiming to find out the least mean value for each RP as well as allowing highlighting the best experimental condition among all tested MSP treatments. According to the Tukey test, the best result for the permeate flux (136.38 kg $m^{-2}$ $h^{-1}$), among all tested MSP conditions, was attained by using a membrane with pore diameter of 0.05 μm under a pressure of 1 bar, at 40 °C (see Table III). Regarding the same experimental condition, grape pulp sample in nature without a previous enzymatic treatment was also tested, exhibiting undoubtedly a lower mean permeate flux (65.16 kg $m^{-2}$ $h^{-1}$). An enzymatic treatment prior to Membrane Separation Process becomes an important pretreatment step, allowing reducing the impact of insoluble particles and suspended solids onto membrane interface with an increasing on the MSP performance along with a suitable clarified grape juice. At least one of set of operating variables has affected significantly

the RP values. Nonetheless, taking altogether, a reduction on the RP values was attained by using the membrane of 0.05 μm, under a pressure of 1 bar, regardless of temperature. With regard to the mean permeate flux and physico-chemical parameters, the best operating condition was verified when using the membrane of 0.05 μm, at 40 °C, under the pressure of 1 bar.

By using the clarified grape juice, originating from the ultrafiltration (0.05 μm, 40 °C, 1 bar), in the reverse osmosis module, operated at a pressure of 40 bar, the permeate flux exhibited values of 8.51 and 4.65 kg $m^{-2}$ $h^{-1}$ for temperatures of 30 and 40 °C, respectively, suggesting that low temperatures are recommended to be used for improving the permeate flux. In addition, the quality of the concentrated grape juice was characterized by the set of five physico-chemical parameters. For a temperature of 30 °C, values of 22.4 g tartaric acid per 100 mL of grape juice, 18.4 °Brix, 10,600 mg Pt-Co $L^{-1}$, 3.62, and 900 FAU were obtained for titratable acidity, soluble solids, color, pH, and turbidity, respectively. Meanwhile for a temperature of 40 °C, values of 23.8 g tartaric acid per 100 mL of grape juice, 20.4 °Brix, 13,000 mg Pt-Co $L^{-1}$, 3.43, and 780 FAU were obtained for titratable acidity, soluble solids, color, pH, and turbidity, respectively. Two grape juice characteristics have been improved at temperature of 40 °C, showing an increasing of the soluble solids and a reduction on the turbidity, reinforcing also the color.

In comparison, the value of soluble solid of grape juice that was pretreated with enzymes followed by two Membrane Separation Processes is twice above those values attributed to three Brazilian commercial grape juices (see Table V). In addition, another positive characteristic was a lower turbidity value than that for commercial grape juices. As a whole, an enzymatic treatment along with micro/ultrafiltration and reverse osmosis has shown a great performance on the production of concentrated grape juice.

**TABLE V**

**Mean values of physico-chemical parameters for non-treated grape pulp (NTGP), enzymatically treated grape pulp (ETGP), permeate grape juice (PGJ) coming from an ultra filtration process, concentrated grape juice (CGJ) coming from an reverse osmosis process, as well as three Brazilian commercial concentrated grape juices (CCGJ).**

| Parameter | NTGP | ETGP | PGJ | CGJ | $CCGJ_1$ | $CCGJ_2$ | $CCGJ_3$ |
|---|---|---|---|---|---|---|---|
| Titratable acidity (g tartaric acid/100 mL sample) | 13.90 | 12.82 | 12.1 | 23.8 | 9.27 | 11.35 | 10.47 |
| Soluble solids (°Brix) | 11.0 | 7.4 | 8.8 | 20.4 | 9.0 | 13.5 | 13.5 |
| Color (mg Pt-Co/L) | 31,550 | 25,900 | 3,000 | 13,000 | 25,400 | 16,800 | 19,600 |
| pH | 3.10 | 3.40 | 3.03 | 3.43 | 3.06 | 3.03 | 3.18 |
| Turbidity (FAU) | 8,700 | 4,550 | 155 | 780 | 4,500 | 2,900 | 3,500 |

## CONCLUSIONS

According to the assessment of enzymatically treated grape pulp characteristics by using the Tukey test an ANOVA, the Novozym 33095 enzyme has shown better result on response physic-chemical parameters than Ultrazym AFP L® one as experiments were performed at 35 °C pectolytic activity temperature, 15 min. treatment time and 50 $mgL^{-1}$ enzyme concentration. The permeate flux of enzymatically treated-grape juice, which were submitted to micro/ultra filtration processes, has shown a behavior depending on the pressure, pore size and temperature. The pressure effect on permeate flux has become more evident on high pressures. A decay on the permeate flux is started with a cake layer formation at 1 bar pressure (0.05 μm) and ended with a complete blocking of membrane pores at 3 bar (0.2 μm), according to a tested fouling mechanism model. An increasing on

the temperature has caused an improvement on the permeate flux due to a reduction on the medium viscosity. High temperature could also contribute to reinforce the fouling mechanism, blocking easily small membrane pores. Nonetheless, the best performance of the MSP with high permeate flux value and suitable grape juice characteristics was attained using 0.05 μm membrane pore size, 1 bar pressure and 40 ºC treatment temperature. An improving on the grape juice characteristics was observed at 40 ºC, with an increasing on the amount of soluble solids and a reduction on the turbidity, reinforcing thus the color. Based on an enzymatic pretreatment of grape pulp followed by MSP, desirable characteristics of the processing grape pulp could be maintained, undesirable characteristic could be reduced by microfiltration and others could be reinforced by reverse osmosis, suggesting that this is an alternative and potential grape juice processing system for application on other types of foods.

**ACKNOWLEDGMENTS**

The authors thank to Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) and Araucaria Foundation for the financial support.

**RESUMO**

Neste trabalho, o melhoramento físico-químico das características do suco de uva concentrado utilizando um tratamento enzimático seguido pelo Processo de Separação de Membranas (PSM) foi investigado. Usando-se as enzimas Novozym ® 33095 e Ultrazym APPL ® variando três parâmetros operacionais, o melhor resultado das características da polpa de uva foi obtido pela Novozym 33095 ® realizada a 35 °C, 15 min. e 50 $mgL^{-1}$. Nos processos de micro / ultra filtragem depois do pré-tratamento enzimático, o melhor desempenho do PSM com valor do alto fluxo permeado e adequadas características do suco de uva foi alcançado usando membrana com tamanho de poro de 0,05 μm, pressão de 1 bar e temperatura de 40 °C tratamento. Quando o processo de osmose reversa é operado a 40 bar e 40 °C, elevados sólidos solúveis e baixos valores da turbidez são alcançados. Um tratamento enzimático juntamente com PSM mostrou um sistema de processo alternativo e eficiente do suco de uva, podendo ser estendido para outros alimentos.

**Palavras-chave:** suco de uva, tratamento enzimático, filtração micro/ultra, osmose inversa.

**REFERENCES**

AOAC - Association of Official Analytical Chemists. 1990. Official method of analysis, 15th ed., v.2, Washington, DC: Association of Official Analytical Chemists.

Balischi L, Pereira NC, Lima OCM, Barros STD, Damasceno JW and Mendes ES. 2002. Influência do tratamento enzimático sobre as características reológicas e microscópicas da polpa de acerola. Acta Sci Tech 24: 1649-1658.

Barros STD, Andrade CMG, Mendes ES and Peres L. 2003. Study of fouling mechanism in pineapple juice clarification by ultrafiltration. J Membr Sci 215: 213-224.

Belleville MP, Brillouet JM, Fuente BT and Moutounet M. 1990. Polysaccharide effects on cross-flow microfiltration of two red wines with a microporous alumina membrane. J Food Sci 55: 1598-1602.

Belleville MP, Brillouet JM, Fuente BT and Moutounet M. 1992. Fouling colloids during microporous alumina membrane filtration of wine. J Food Sci 57: 396-400.

Chen CS, Shaw PE and Parish ME. 1993. Orange and tangerine juices. In: Fruit Juice Processing Technology, Auburndale: AG Science Inc. Nagy S, Chen CS and Shaw PE (Eds), Auburndale, Florida, USA, p. 110-119.

Czekaj P, López F and Güell C. 2000. Membrane fouling during microfiltration of fermented beverages. J Membrane Sci 166: 199-212.

El Rayess Y, Albasi C, Bacchin P, Taillandier P, Raynal J, Mietton-Peuchot M and Devatine A. 2011. Cross-flow microfiltration applied to oenology: A review. J Membrane Sci 382: 1-19.

Espinoza-Quiñones FR, Módenes AN, Thomé LP, Palácio SM, Trigueros DEG, Oliveira AP and Szymanski N. 2009. Study of the bioaccumulation kinetic of lead by living aquatic macrophyte *Salvinia auriculata*. Chem Eng J 150: 316-322.

Field RW, Wu D, Howell JA and Gupta BB. 1995. Critical flux concept for microfiltration fouling. J Membrane Sci 100: 250-272.

Giorno L, Todisco S, Donato L and Driolo E. 1998. Study of fouling phenomena in apple juice clarification by enzyme membrane reactor. Separ Purif Technol 33: 739-756.

Girard B and Mazza G. 1998. Functional grape and citrus products. In: Functional Foods: Biochemical and

Processing Aspects. Mazza G (Ed), The Free Library, Lancaster, PA, USA, p. 139-191.

GONZÁLEZ-BARRIO R, VIDAL-GUEVARA ML, TOMÁS-BARBERÁN FA AND ESPÍN JC. 2009. Preparation of a resveratrol-enriched grape juice based on ultraviolet C-treated berries. Innov Food Sci Emerg Technologies 10: 374-382.

GURAK PD, CABRAL LMC, ROCHA-LEÃO MHM, MATTA VM AND FREITAS SP. 2010. Quality evaluation of grape juice concentrated by reverse osmosis. J Food Eng 96: 421-426.

HABERT AC, BORGES CP AND NÓBREGA R. 2006. Processos de Separação por Membranas, Rio de Janeiro: Ed. e-papers, 180 p.

HÉRMIA J. 1982. Constant pressure blocking filtration laws. Applications to power – law non – Newtonian fluids. T I Chem Eng-Lond 60: 183-187.

JANG M, CAI L, UDEANI GO, SLOWING KV, THOMAS CF AND BEECHER CWW. 1997. Cancer chemopreventive activity of resveratrol, a natural product derived from grapes. Science 275: 218-220.

JERMANN D, PRONK W, KÄGI R, HALBEISEN M AND BOLLER M. 2008. Influence of interactions between NOM and particles on UF fouling mechanisms. Water Res 42: 3870-3878.

JIAO B, CASSANO A AND DRIOLI E. 2004. Recent advances on membrane processes for the concentration of fruit juices: A review. J Food Eng 63: 303-324.

KASHYAP DR, VOHRA PK, CHOPRA S AND TEWARI R. 2001. Applications of pectinases in the commercial sector: a review. Bioresource Technol 77: 215-227.

KATSOUFIDOU K, YIANTSIOS SG AND KARABELAS AJ. 2005. A study of ultrafiltration fouling by humic acids and flux recovery by backwashing: experiments and modeling. J Membrane Sci 266: 40-50.

KÖSEÓGLU SS, LAWHON JT AND LUSAS EW. 1990. Use of membranes in citrus juice processing. Food Technol 44: 90-97.

LEE WC, YUSOF S, HAMID NSA AND BAHARIN BS. 2006. Optimizing conditions for enzymatic clarification of banana juice using response surface methodology (RSM). J Food Eng 73: 55-63.

MERSON RL, PAREDES G AND HOSAKA DB. 1980. Concentrating fruit juices by reverse osmosis. In: Ultrafiltration Membranes and Applications. Cooper AR (Ed), New York: Plenum Publishing, 405 p.

MÓDENES AN, ESPINOZA-QUIÑONES FR, TRIGUEROS DEG, PIETROBELLI JMTA, LAVARDA FL, RAVAGNANI MASS AND BERGAMASCO R. 2012. Binary Adsorption of a Zn(II)-Cu(II) Mixture onto *Egeria densa* and *Eichhornia crassipes*: Kinetic and Equilibrium data modeling by PSO. Separ Science Technol 47: 875-885.

NOVOZYMES MANUAL. 2001. Product Sheet, Fruit & Vegetable-07212-02. Araucária: Novozymes Latin America Limited.

PÉREZ-LAMELA C, GARCÍA-FALCÓN MS, SIMAL-GÁNGARA J AND ORRIOLS-FERNÁNDEZ IJ. 2007. Influence of grape variety, vine system and enological treatments on the colour stability of young red wines. Food Chem 101: 601-606.

PETER-VARBANETS M, MARGOT J, TRABER J AND PRONK W. 2011. Mechanisms of membrane fouling during ultra-low pressure ultrafiltration. J Membrane Sci 377: 42-53.

QU F, LIANG H, WANG Z, WANG H, YU H AND LI G. 2012. Ultrafiltration membrane fouling by extracellular organic matters (EOM) of *Microcystis aeruginosa* in stationary phase: Influences of interfacial characteristics of foulants and fouling mechanisms. Water Res 46: 1490-1500.

RAI P, MAJUMDAR GC, DASGUPTA S AND DE S. 2004. Optimizing pectinase usage in pretreatment of mosambi juice for clarification by response surface methodology. J Food Eng 64: 397-403.

SACCHI KL, BISSON LF AND ADAMS DO. 2005. A review of winemaking techniques on phenolic extraction in red wines. Am J Enol Viticult 56: 197-206.

SIN HN, YUSOF S, HAMID NSA AND RAHMAN RA. 2006. Optimization of enzymatic clarification of sapodilla juice using response surface methodology. J Food Eng 73: 313-319.

THOMASSET SC, BERRY DP, GARCEA G, MARCZYLO T, STEWARD WP AND GESCHER AJ. 2006. Dietary polyphenolic phytochemicals. Promising cancer chemopreventive agents in human? A review of their clinical properties. Int J Cancer 120: 451-458.

TRIGUEROS DEG, MÓDENES AN, ESPINOZA-QUIÑONES FR AND KROUMOV AD. 2010b. The evaluation of benzene and phenol biodegradation kinetics by applying non-structured models. Water Sci Technol 61: 1289-1298.

TRIGUEROS DEG, MÓDENES AN, ESPINOZA-QUIÑONES FR AND RAVAGNANI MASS. 2012. Reuse Water Network Synthesis by Modified PSO Approach. Chem Eng J 183: 198-211.

TRIGUEROS DEG, MODENES AN, KROUMOV AD AND ESPINOZA-QUIÑONES FR. 2010a. Modeling of biodegradation process of BTEX compounds: kinetic parameters estimation by using Particle Swarm Global Optimizer. Process Biochem 45: 1355-1361.

TODISCO S, PEÑA L, DRIOLI E AND TALLARICO P. 1996. Analysis of the fouling mechanism in microfiltration of orange juice. J Food Process Preserv 20: 453-466.

VAILLANT F, MILLAN A, DORNIER M, DECLOUX M AND REYNES M. 2001. Strategy for economical optimisation of the clarification of pulpy fruit juices using crossflow microfiltration. J Food Eng 48: 83-90.

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. J Food Eng 42: 215-224.