Evaluation of anthocyanin content on blackberry juice (*Rubus* spp.) processed by microfiltration

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ABSTRACT

Small red fruits have been attracting the attention of producers and consumers, especially after news reports about the benefits they provide to the human health due to the presence of high content of phenolic compounds with antioxidant capacity. Besides being a good source of natural antioxidants, vitamins and minerals, blackberries are rich in phenolic acids, flavonoids, including anthocyanins, conferring to the juice a highly attractive colour, but also a instability to some severe heat treatments, which can result in darkened and cooked taste products. The target is to process the initial raw materials by less aggressive techniques. This study aimed to evaluate the effect of microfiltration process on the anthocyanins content from blackberry juice. The juice was obtained from pressing blackberry fruits in a depulper machine with a 0.8 mm sieve. Before microfiltration, the juice was centrifuged and submitted to an enzymatic hydrolysis for 30 min with 400 ppm of pectic enzyme at 35 °C in order to reduce the insoluble solids content, the viscosity and hence increase the permeate flux during microfiltration. Microfiltration was carried out in a plate and frame module with fluorinated membranes of 0.15 micron pore size and total permeation area of 0.36 m^2 at 35°C and transmembrane pressure of 5 bar. The retentate stream was recirculated back to the feed tank and the permeate was continuously collected. The average permeate flux was 22 L/hm² and the volumetric concentration factor reached 7. The anthocyanin content of the single strength blackberry juice was 46 mg/100g and of the permeate juice 43 mg/100g, proving the effectiveness of the microfiltration process for the maintenance of the anthocyanin concentration of the blackberry juice. These results motivate the continuity of new researches on the application of membrane technology to fruit juice processing.

Keywords: blackberry juice; anthocyanins; membrane technology; clarification.

INTRODUCTION

Human diet can act as a factor in disease prevention and it is considered a relevant factor for a healthy lifestyle. The consumption of fruits and vegetables has been associated with the prevention of several types of diseases due to presence of substances, especially phenolic compounds that present an antioxidant capacity. Blackberry (*Rubus* spp.), besides being a good source of natural antioxidants, vitamins and minerals, is rich in phenolic acids, flavonoids, including anthocyanins, which give a highly attractive color to this fruit. Several studies show that anthocyanic pigments have anticarcinogenic activity [1, 2], antioxidant [3] and antiviral [2] promoting an association of these properties to foods that contain them.

Due to the fragile structure and high respiratory rate, blackberry fruits have a short shelf-life. They are mainly commercialized as processed products like frozen fruits, canned, pulp, juice, jellies, syrups.

Nowadays there is a high demand for clarified juices from the industries of beverages and refreshments, ice-creams and jellies. The clarification of fruit juices is often constituted by a series of processes and operations that attempt to remove the components responsible for the turbidity and instability of the product. A limpid and stable product must be obtained presenting in general a smaller viscosity than the single strength juice. Blackberry juice is unstable to severe treatments, which can result in a darkened and altered taste product [4]. Membrane separation processes represent a potential alternative to the preservation and concentration of fruit juices. These processes are usually carried out at room temperature under mild conditions, thus avoiding the oxidation and degradation of thermolabile compounds and minimizing the quality loss of the products [5]. The consumers' interest for healthier and natural products has been growing and contributing to the consumption of fruit juices and based fruit beverages.

This work aims at to evaluate the effect on the anthocyanin content of the clarification of blackberry juice (*Rubus* spp.) by microfiltration.

MATERIALS & METHODS

Frozen blackberries (*Rubus* spp.) were used as raw material. The fruits were processed in a pulper machine with a 0.8 mm sieve. The obtained juice was then centrifuged in a basket centrifuge at 4,000 rpm (479.2 G) and stored at -17°C until use.

Microfiltration was carried out in a plate and frame module (Lab Unit M20 – Gea Filtration) with ten flat sheet fluorinated membranes of 0.15 µm pore size and total permeation area of 0.36 m^2. The processes were conducted in a batch mode with recirculation of the retentate fraction to the feed tank and continuous collection of the permeate stream. Transmembrane pressure was maintained at 5 bar (maximum suggested by the manufacturer) and the temperature was kept at 35°C.

Before microfiltration, the centrifuged juice was enzymatically treated with 400 ppm of Rapidase® at 35°C for 30 minutes.

The process was performed in triplicate. Samples were taken from all the fractions (feed, permeate and retentate) to be submitted to the following analysis:

1- Titratable acidity was determined in a titrator Metrohm 785 DMP Titrino with sodium hydroxide reagent [6];
2- Measurements of pH were performed in automatic titrator Metrohm® (model 785 DMP – Titrino) after calibration of the device with buffers of pH 4,00 and 7,00 [6];
3- Soluble solids content was determined by direct reading in a refractometer model Bellingham + Stanley Limited with temperature correction (20ºC) and expressed in ºBrix [6];
4- Total solids content was determined in a vacuum oven at 65 ° C until constant weight, according to AOAC [6];
5- Analyses of total and monomeric anthocyanins were held in spectrophotometer at 520 nm, according to the differential pH method described by Giusti and Wrolstad [7];
6- The antioxidant activity was determined by spectrophotometry, using the free radical $ABTS^{\bullet +}$ decolorization assay according Re *et al*. [8].

RESULTS & DISCUSSION

The behavior of the permeate flux along microfiltration of the blackberry juice is showed in Figure 1. It can be verified that the permeate flux markedly decreased at the first 15 minutes of the permeation. The initial permeate flux (± 43L/hm^2) decreased to 20L/hm^2 remaining constant to the end of the process. The three repetitions of the process (MF1, MF2, MF3) had the same flux behavior. One of the major limiting factors in membrane processes is the decay of the permeate flux during the processing which is related to the phenomena of concentration polarization and fouling.

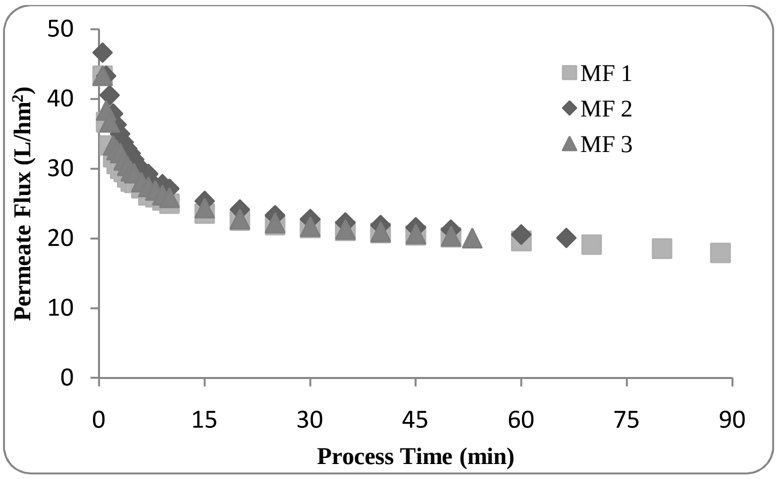

Figure 1. Permeate flux of blackberry juice along microfiltration of blackberry juice (process carried out in triplicate).

According Cheryan [9], solids are dragged into the membrane surface by convective transport and they are partially or totally rejected, and tend to concentrate on the interface, forming a concentration gradient. This increase in concentration gives the name of polarization by concentration. In juice processing, compounds such as pectins, cellulose, hemicellulose, starch, and proteins cause undesired turbidity during storage and the

juice thus has to be clarified. Membrane fouling has been widely studied for membrane researchers since it reduces the productivity and membrane life [9, 10]. According to Marshall and Munro [10], the decline of the permeate flux can be divided in three steps. First, it is evidenced the result of polarization by concentration, then, adsorption phenomena and at last the deposition of the fouling.
It was verified that the volumetric concentration factor (VCF - defined as the ratio between the initial feed volume and the volume of the resulting retentate) increased exponentially along processing (Figure 2).
The three processes carried out under the same conditions shown different curves of the course of VCF. It must be pointed out that the triplicates were conducted with different initial juice volumes so that they had different processing time because VCF was maintained fixed. The volume of the permeated juice increased linearly along the microfiltration (Figure 3).

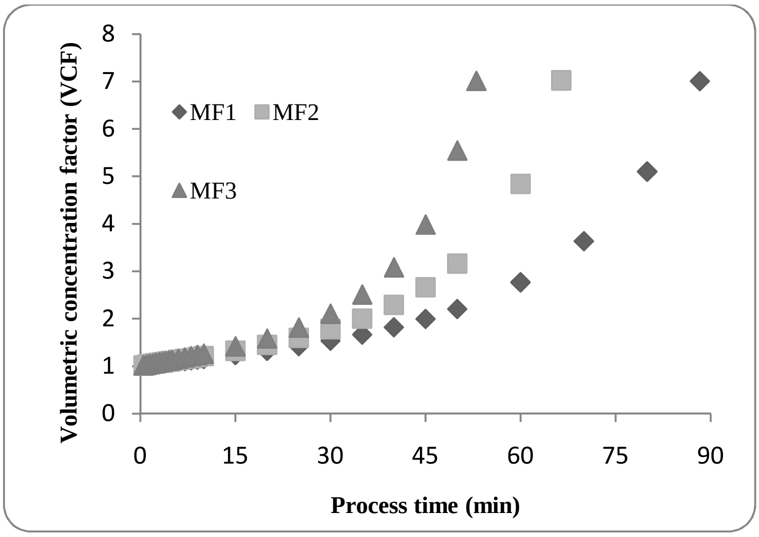

Figure 2. VCF along microfiltration of blackberry juice

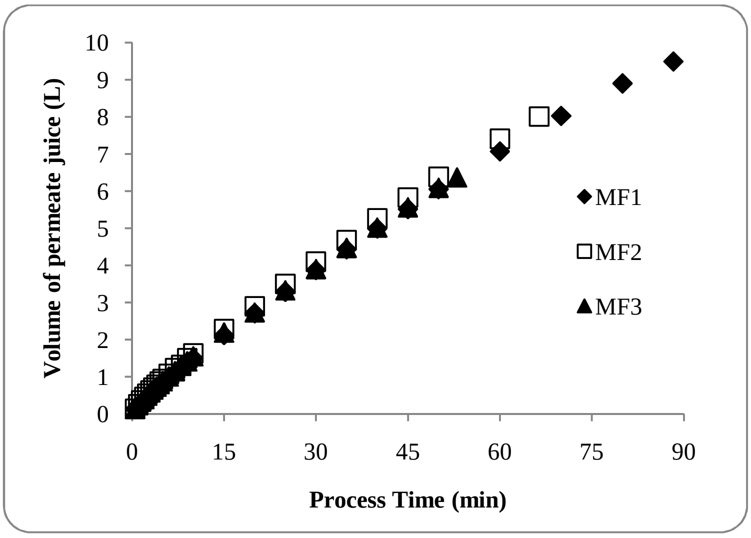

Figure 3. Cumulative volume of permeate juice along microfiltration of blackberry juice

Anthocyanin content of the single strength blackberry juice was 46 mg/100g and of the permeate juice 43 mg/100g, proving the effectiveness of the microfiltration process for the maintenance of the nutritional quality of the blackberry juice (Table 1).

Regarding the antioxidant capacity, it was found a reduction of approximately 25% in the clarified juice when compared to the single strength juice. Besides, the retentate fraction increased suggesting that other substances that also contribute for the total antioxidant capacity are been retained by the membrane (Table 2). As showed in Table 3, the physico-chemical parameters of the products did not present significant variation in the three different experiments.

Table 1. Anthocyanin content of blackberry juice processed by microfiltration

	Anthocyanin (mg/100g)			
	MF_1	MF_2	MF_3	Average
Feed	47.29 ± 0.13	46.67 ±0.31	46.20 ±0.18	46.67 ± 0.54
Permeate	45.00 ± 0.17	43.74 ±0.12	43.23 ±0.10	43.74 ± 0.91
Retentate	60.59 ± 0.05	60.70 ±0.20	60.63 ±0.23	60.63 ± 0.05

Table 2. Antioxidant capacity of blackberry juice processed by microfiltration

	Antioxidant Capacity (μmol trolox/g)			
	MF_1	MF_2	MF_3	Average
Feed	10.13 ± 0.09	10.14 ±0.03	10.26 ±0.15	10.14 ± 0.07
Permeate	7.85 ± 0.04	7.60 ±0.02	7.29 ±0.04	7.60 ± 0.28
Retentate	19.87 ± 0.20	19.70 ±0.09	18.82 ±0.10	19.70 ± 0.57

Table 3. Characteristics of blackberry juice processed by microfiltration

	Feed	**Permeate**	**Retentate**
Acidity (g/100g citric acid)	1.14 ± 0.02	1.12 ± 0.02	1.34 ± 0.00
pH	3.08 ± 0.03	3.06 ± 0.03	3.09 ± 0.03
Total soluble solids (°Brix)	7.00 ± 0.29	7.00 ± 0.00	9.00 ± 0.29
Total solids (g/100g)	8.33 ± 0.71	7.43 ± 0.24	9.79 ± 0.34

CONCLUSION

The obtained results indicate that microfiltration can be applied to clarify blackberry juice maintaining the anthocyanin content in both streams, retentate and permeate, motivating the continuity of new researches on membrane technology to fruit juice processing.

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