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Concentration and purification by crossflow microfiltration with diafiltration of carotenoids from a by-product of cashew apple juice processing

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Abstract

This work aimed to further investigate microfiltration with diafiltration to obtain a rich carotenoid extract from the solid by-product of cashew apple (\textit{Anacardium occidentale} L.) juice processing. This solid residue called cashew fibers is rich in bioactive compounds, especially carotenoids. After mixing with water and pressing, an aqueous suspension was obtained and processed by crossflow microfiltration (40°C) using tubular ceramic membrane with 0.2 \mu m pore diameter. Permeate flux, which increased with transmembrane pressure, could be maintained above 100 L·h\textsuperscript{-1}·m\textsuperscript{-2} at high volume reduction ratio by coupling mechanical extraction with enzyme liquefaction. The concentration step led to an increase of the carotenoid content by up to 19-fold and the diafiltration step allowed the carotenoid purity to be multiplied by 5. The impact of the process on the retentate characteristics was assessed using a model based on simple assumptions. This calculation tool can easily be implemented and is helpful for choosing the operating conditions, to minimize water consumption as well as effluent production.

Industrial relevance text

A driven pressure membrane operation is a mild and economic process which covers a broad variety of applications in the food industry. Most operations focus on permeate (clarification, cold sterilization, etc.), however few attempts have been made to exploit the retentate which is considered as waste in many cases. In this study, we devised an approach for an integrated process of extraction and crossflow filtration combined with enzymatic liquefaction in order to produce a carotenoid-rich extract from cashew apple fibers. This work also provided opportunities for cashew apple juice manufacturers to add value to their by-products at low cost, simultaneously producing a high value-added extract and a ready-to-drink clarified juice. Another originality of this work was to provide a simple equation set, accessible on an industrial scale, to forecast the effluent volume and the consumption of water during diafiltration, by setting a target concentration factor and a target purification factor, correlated to the limit of the purification rate. This work can help industrials to plan production.
providing them with the possibility of steering the process with a balance between final product quality, process productivity, effluent generation and water consumption.

**Highlights**

Integrated extraction-filtration process of cashew fibers with enzyme liquefaction

Total carotenoid retention during concentration and purification by microfiltration

High permeate flux at a volume reduction ratio of up to 20

Carotenoid purity multiplied by 5 using diafiltration

Modelling to forecast purification rate minimizing effluent volume and water consumption

**Keywords**: microfiltration efficiency, purification rate, diafiltration performance, carotenoid rejection, enzyme liquefaction

**Abbreviations**

α: purification rate (no unit)

CF: concentration factor (no unit)

C: carotenoid concentration (mg·kg⁻¹)

DM: dry matter (g·kg⁻¹)

DVR: diavolume ratio (no unit)

Jₚ: permeate flux (L·h⁻¹·m⁻²)

p: purity of carotenoids (mg·kg⁻¹)

PF: purification factor (no unit)

R: retention rate (no unit)

SIS: suspended insoluble solids (g·kg⁻¹)

TmP: transmembrane pressure (bar)

TSS: total soluble solids (g·kg⁻¹)

VRR: volume reduction ratio (no unit)

V: volume (L)

Subscripts 0, r, p refer to initial, retentate and permeate respectively
1. Introduction

Over the last decades, crossflow microfiltration, and more broadly pressure-driven membrane processes, have been further developed for the fruit juice industry for multiple operations including clarification, sterilization, concentration or fractionation (Conidi et al., 2020; Dornier et al., 2018; Lipnizki, 2010). Crossflow microfiltration involves suspensions and is performed to separate insoluble compounds (particles, droplets, colloids) using 0.1 to 10 µm pore diameter membranes while ultra- and nanofiltration are used for solute fractionation over a large molecular weight range. Crossflow microfiltration under 0.2 µm pore diameter is mainly carried out as a unit operation throughout the technology chain in the food industry. This mild process is often used to decrease the particle content in a liquid (clarification), as a pre-treatment to prevent any problems with subsequent operations (chromatographic separation, other membrane processes, etc.), and to lower the microbial load in the permeate (Dornier et al., 2018). For industrial applications, multiple parameters should be considered including environmental, economic or technological ones. Regarding the process, key parameters such as permeate flux, yields and rejections have to be evaluated and optimized to estimate the large-scale feasibility. Microfiltration is often associated with an enzymatic treatment which aims to reduce viscosity and fouling, and in most cases, to improve the system performance (De Oliveira et al., 2012; Laorko et al., 2010; Vaillant et al., 2005). Since the enzymes used are a mix of pectinases, cellulases and hemicellulases from mold cultures which are commonly used in the fruit processing industry, the cost of such processing aids is generally low, compared to the productivity gain. Most studies of membranes carried out in various fields, have generally focused on enhancing the value of permeate while only a few have highlighted retentate potential. For their part, they mainly describe the concentration and the purification of bioactive compounds, associated with the comprehension of fouling mechanisms in complex matrices (Cho et al., 2003; Cisse et al., 2011; Dahdouh et al., 2016).
Cashew apple (*Anacardium occidentale* L.) is cultivated on a large scale, especially in Brazil which ranked 1st in 2018 producing more than 1 500 000 tons according to FAO statistics. It is associated with cashew nut production, which is the main reason why it is produced, however it suffers from considerable post-harvest losses (Das & Arora, 2017). It is associated with cashew nut production, which is the main reason why it is produced. It has been reported to be rich in organic acids, sugars, polysaccharides, mostly pectin, and in phytochemicals, such as polyphenols, mainly flavonoids and tannins, carotenoids and vitamins, especially vitamin C. Like all fruits, it is highly perishable, and spoilage of the pulp occurs rapidly, only a few hours after fruit injury or during the first step of juice processing. The type of spoilage observed is usual, namely fermentation and oxidation leading mainly to browning and astringency development (Das & Arora, 2017). Cashew apple that is intended for human consumption, is processed into multiple products such as sweet foods (candies, jam, etc.) or beverages. This industrial production leads to the rejection of waste in significant quantities which is named cashew apple fibers (Damasceno et al., 2008; Talasila & Shaik, 2015). This by-product, mainly destined for animal food production, is however rich in bioactive compounds, especially carotenoids. Carotenoids are liposoluble compounds that have been studied for years because of their role as colorants and antioxidants. In addition to the pro-vitamin A status of some of them, they present known and significant beneficial effects on human health, for instance, reducing the risk of cancer, cardiovascular disease and cellular degeneration (Rao & Rao, 2007). Pinto de Abreu et al. (2013), have characterized the carotenoids and their relative contents in cashew apple fibers. The carotenoids identified were mainly xanthophylls, such as auroxanthin, mutatoxanthin, lutein, zeaxanthin and β-cryptoxanthin at 31, 24.5, 10.4, 11 and 15% of total carotenoids respectively, but also carotenes, mostly β-carotene, at 7.4%. In this work, the authors highlighted an approach using pressing and enzyme treatment in order to optimize the extraction of carotenoids from cashew
fibers. A strategy based on a continuous helical-type press was suggested for optimal carotenoid extraction, and a microfiltration trial was mentioned in order to obtain a concentrated extract. Campos et al. (2002) and Castro et al. (2007) presented interesting results using this strategy, demonstrating the feasibility of crossflow microfiltration applied to cashew apple juice to decrease the microflora in the clarified permeate.

Crossflow microfiltration coupled with enzyme liquefaction, resulted in two promising derived products with significant process performance. In the case of melon juice, Vaillant et al. (2005) obtained on one hand a retentate enriched 4-fold in β-carotene, and on the other a clarified permeate which was organoleptically close to fresh melon juice. Introducing a diafiltration step allows the purity of the obtained fractions to be increased. For watermelon, integrated processes combining crossflow microfiltration and enzyme liquefaction have been developed for the production of a bioactive lycopene extract, concentrated between 8 and 11-fold and purified up to 25-fold by diafiltration (Chaparro et al., 2016) or between 12 and 18-fold, resulting in a higher antioxidant capacity of the extract (Oliveira et al., 2016). Using in vitro human digestion models, Gence et al. (2018) highlighted the nutritional interest of a clementine juice concentrated 8-fold by crossflow microfiltration, taking into account carotenoid bioaccessibility. This research also underlined the benefit of considering the retentate as a high-value product, both economically and nutritionally.

Recently, a study was performed in order to assess the feasibility of coupling crossflow microfiltration and diafiltration for carotenoid concentration and purification from orange juice using an industrial approach (Polidori et al., 2018). In addition to the production of a concentrated carotenoid extract between 5 and 10-fold which was purified over a range of 8 to 20-fold by diafiltration with relevant process performances, the authors provided and validated a model based on simple assumptions in order to predict concentration and purification factors according to targeted conditions or vice versa on any filtration scale.
The present work aims to study the microfiltration process that includes a diafiltration step for concentration and purification of carotenoids in an aqueous extract obtained from cashew apple fibers. It includes an evaluation of the interest of coupling the process with enzymatic liquefaction. The study proposes to address the operation integrating product quality, process productivity, and part of the environmental impact. A special focus is placed on developing a simple simulation tool for helping the user to choose operating conditions that result in the targeted concentration and purification levels minimizing effluent generation and processing duration.

2. **Experimental**

2.1 **Cashew juice material**

Raw material obtained from the by-product of cashew apple juice production as described by Pinto de Abreu et al. (2013), was supplied by Sabor Tropical Ltda (Ceará, Brazil) and was produced from the CCP-076 variety of cashew apple developed by Embrapa. This by-product, called cashew apple fibers, was mixed with water using a mass ratio of 1:1. At this stage, maceration for 60 min at 55°C with 500 mg·kg$^{-1}$ of pectinolytic enzyme Ultrasim AFP-L (Novozymes, Bagsvaerd, Denmark) was, in certain cases, carried out. This aimed to improve the release of carotenoids that are strongly entrapped within cell structures. The resulting mixture was then pressed in a helical-type press with a nominal capacity of 300 kg·h$^{-1}$ using a rotational speed of 30 rpm (Incomat 300, Fortaleza, Brazil). This operation was repeated 6 times after remixing the two phases obtained. The final extract was filtered through a 0.3 mm sieve. For the study, one homogeneous batch of around 230 L of final extract was constituted, distributed in sealed 3-10 L polyethylene bags and frozen at -20°C until use.

2.2 **Microfiltration process: clarification & purification**
Microfiltration trials were carried out at laboratory scale using a micro-pilot manufactured by TIA (Bollène, France). This device is described in figure 1. It consisted of 4 single-channel tubular α-alumina membranes of 55 cm² each, with an average pore diameter of 0.2 µm, mounted in series (Pall Exekia, Bazet, France). This configuration allowed the simultaneous testing of 4 transmembrane pressure (TmP) conditions ranging from 1.2 to 3.2 bar. For each membrane TmP was evaluated from the input and output pressures in the retentate loop considering head losses as linear. Crossflow velocity was set at around 6 m·s⁻¹ independently of TmP using a volumetric feed pump. This high value ensured that the system acted as a perfectly stirred reactor. The temperature of the whole system was maintained at 40 ± 2°C using a heat exchanger connected to a running water cryostat. This pilot was equipped with a feeding tank of 3 L. Membrane cleaning operations were based on manufacturer recommendations, including classical alkaline (NaOH 2 %, 80°C, 20 min without pressure, 20 min with pressure) and acidic phases (HNO₃ 1 %, 50°C, 20 min), and was controlled by the verification of pure water permeability (374 ± 103 L·h⁻¹·m⁻²·bar⁻¹ at 25°C and 6 m·s⁻¹).

As usual, the concentration level of the extract was characterized by the volume reduction ratio (VRR) defined according to the volumes of permeate $V_p$ and retentate $V_r$ (Equation 1).

$$VRR = \frac{V_r + V_p}{V_r} = 1 + \frac{V_p}{V_r}$$  

Each filtration was carried out in concentration mode extracting the permeate continuously. Starting with 2.5-3 L of the product, the system was continuously fed with fresh raw extract, maintaining the retentate volume constant (concentration at constant volume) up to VRR between 5 and 8. Then, in order to reach a higher VRR more quickly, feeding was stopped, allowing the volume of the retentate to decrease (concentration at variable volume). At the end of the concentration step, the $V_r$ was still 1.5 L. During filtration, the retention rate (R) is
defined using Equation 2 comparing the concentrations in the permeate \( C_p \) and in the retentate \( C_r \) as usual.

\[
R = 1 - \frac{C_p}{C_r} \quad (2)
\]

A diafiltration step could then be added at the end of the concentration step when the targeted VRR was reached. In this case, diafiltration was carried out at constant \( V_r \) value (1.5 L) by feeding the system with distilled water instead of fresh raw extract in order to compensate the volume of permeate recovered. Therefore, the diafiltration was continuous, without interruption between the concentration and the diafiltration steps. This diafiltration mode allowed water consumption to be minimized because it was carried out at the end of the concentration step when \( V_r \) was the lowest (Polidori et al. 2018). Diafiltration ended when permeate reached a content of total soluble solids below 5 g·kg\(^{-1}\). The diavolume ratio (DVR) was defined as the ratio between the volume of added water \( V_w \) and \( V_r \) (Equation 3). It can also be considered as a water consumption indicator.

\[
DVR = \frac{V_w}{V_r} \quad (3)
\]

### 2.3 Extract analysis

Total soluble solids (TSS) were measured with a PAL-\( \alpha \) refractometer (Atago, Tokyo, Japan) with ± 0.5 g·kg\(^{-1}\) of bias. To assess the suspended soluble solids (SIS), a sample of precisely 20 g was homogenized and centrifuged at 14 000 x g for 20 min (Allegra 21 centrifuge, Beckman Coulter, USA). The supernatant was discarded and replaced by 20 g of deionized water and then the sample was homogenized and centrifuged again. After 3 repetitions, the residue was dried at 70°C under vacuum (100 mPa) and weighted. The term dry matter (DM) refers to the residues remaining after the total evaporation of water. DM includes both TSS and SIS. Therefore, it was calculated as the sum of these two fractions.
To quantify the total carotenoid content, 10 mL of the sample to be analyzed were homogenized with 35 mL of a mixture made of ethanol/hexane (4:3 v/v) (Sigma-Aldrich, Santa-Clara, USA). After dephasing in a separating funnel, the organic phase was recovered, dried with anhydrous sodium sulfate (Sigma-Aldrich, Santa Clara, USA) and its absorbance was then measured at 450 nm with a Variant 50 Bio spectrophotometer (Variant Inc, California, USA). Results were expressed in mg $\beta$-carotene equivalent using the molar extinction coefficient of $\beta$-carotene at 450 nm. Carotenoid purity $p$ was calculated as carotenoid content expressed in DM. All analyses were carried out in triplicates.

2.4 Modelling

Assuming that there were no losses of matter during the operation, that the system behaved like a perfectly stirred reactor and that the retentions were constant, the retentate composition could be predicted using a simple equation set tested in previous studies (Acosta et al., 2014; Polidori et al., 2018). Indeed, since the insoluble compounds (SIS and carotenoids) were completely retained by the membrane, unlike the TSS, which were not retained at all (Chaparro et al., 2016; Oliveira et al., 2016; Polidori et al., 2018), carotenoid concentration ($C$), SIS and TSS could be calculated in the final retentate from volume reduction ratio (VRR), diavolume ratio (DVR) and initial values (subscript 0) using Equations 4 to 6.

\[
C = C_0 \times VRR \quad (4)
\]

\[
SIS = SIS_0 \times VRR \quad (5)
\]

\[
TSS = TSS_0 \times e^{-DVR} \quad (6)
\]

Therefore, for carotenoids the concentration factor in the retentate (CF) is equal to the VRR.
Since DM is the sum of SIS and TSS, the purity of carotenoids in the retentate p and the purification factor (PF) could be assessed through Equations 7 and 8.

\[
p = \frac{C_0 \cdot VRR}{SIS_0 \cdot VRR + TSS_0 \cdot e^{-DVR}} \quad (7)
\]

\[
PF = \frac{(SIS_0 + TSS_0) \cdot VRR}{SIS_0 \cdot VRR + TSS_0 \cdot e^{-DVR}} \quad (8)
\]

In this case, the purity of carotenoids cannot exceed the ratio \( C_0 / SIS_0 \) when DVR increases.

Thus, whatever the VRR, it is not possible to exceed a maximum purification factor \( PF_{lim} \). In order to quantify the progress of the purification, we defined the ratio between the obtained PF and \( PF_{lim} \) (Equation 9). It ranges from 0 to 1 and was called the purification rate \( \alpha \) (Equation 10).

\[
PF = \alpha \cdot PF_{lim} = \alpha \cdot (TSS_0 / SIS_0 + 1) \quad (9)
\]

\[
\alpha = \frac{VRR}{VRR + (TSS_0 / SIS_0) \cdot e^{-DVR}} \quad (10)
\]

3. Results and discussions

3.1. Effect of transmembrane pressure

In order to find an optimal transmembrane pressure, preliminary trials were carried out using the extract obtained without enzymes (Table 1) up to a VRR of around 10. In figure 2, the permeate flux \( (J_p) \) during microfiltration of cashew apple extract without enzyme liquefaction using 5 growing TmP (from 2.2 to 3.2 bar) were plotted versus VRR. As presented, permeate flux abruptly decreased at the beginning as soon as concentration occurred. This initial phase is usual in crossflow microfiltration and corresponds to a rapid membrane fouling mainly due to accumulation of insoluble solids on the membrane surface (external fouling) and/or into the membrane pores (internal fouling). This fouling leads to an increase in the total hydraulic
resistance of the system and so causes the drop in transmembrane flux. Shortly afterwards, from a VRR of around 1.2, the flux decrease slowed down, what could be linked to back-transport phenomena due to crossflow velocity that counterbalanced the input of fouling material to the membrane. From a VRR = 3, \( J_p \) stabilized up to the final VRR. This behavior was observed irrespective of the transmembrane pressure. By comparison with many references in literature that dealt with crossflow microfiltration of various fruit juices, these last results were different (Emani et al., 2013; Fukumoto et al., 1998; Qin et al., 2015; Ushikubo et al., 2007; Wang et al., 2005). Even if the flux range was in accordance with the values generally mentioned for fruit juice microfiltration (Dornier et al., 2018; Rai & De, 2009), stabilization at such a high VRR is uncommon. Generally transmembrane transfers are continuously slowed down by the increase in viscosity and fouling power of the retentate during concentration (Tarabara et al., 2002). This original behavior was probably linked to the extract composition that was quite different from classical fruit juices in terms of soluble/colloidal/insoluble fractions and led to a more moderate fouling of the membrane during concentration. Indeed, it is well established that fouling not only depends on the hydrodynamic conditions in the vicinity of the membrane but also on suspension characteristics (Dahdouh et al., 2016). Nevertheless, because the phenomena involved in membrane fouling are multiple and complex, further investigations should be carried out for a better understanding. This configuration is particularly favorable for an industrial application as it makes it possible to reconcile high concentration factors and high treatment processing rates, close to 100 L·h\(^{-1}\)·m\(^{-2}\), considered very interesting from an economic point of view for microfiltration of food liquids (Daufin et al., 2001). This promising performance underlined the fact that the fouling property of the product was limited.

The figure 3 represented the stabilized permeate flux \( J_p \) depending on the used TmP (from 2.2 to 3.2 bar) for a range of VRR between 1 to 9. Comparing the permeate flux for the same
VRR, it linearly increased with pressure both at low and high VRR. These results showed that permeate fluxes were mainly impacted by the driving force of the mass transfer through the porous medium (i.e. the transmembrane pressure gradient) with a total hydraulic resistance of the system, defined according to the generalized Darcy’s law, that increased with the volumetric reduction level at low VRR and then tended to a constant value for higher VRR. Therefore, in the chosen operating conditions, high TmP at about 3 bar has to be selected in order to maximize permeate flux whatever the desired targeted concentration factor for carotenoids. These experimental results were significantly different from those obtained by Polidori et al. (2018) with orange juice which showed that the effect of pressure on permeate flux depended on VRR. The much weaker fouling power of cashew apple fiber extract could be linked, as mentioned by Dahdouh et al., (2016); Vaillant et al., (2008) and Tarabara et al., (2002), to multiple factors such as its composition, particle size distribution and rheological characteristics, related to the specific extraction press-processing, but further studies are needed to confirm this.

3.2. Effect of enzymatic liquefaction during extraction

Extracts obtained with and without enzyme treatment exhibited very similar TSS and SIS contents (Table 1). Thus, liquefaction did not drastically modify the soluble/insoluble solid distribution in the final product. On the contrary, carotenoid content was 30% higher in the enzyme-treated product, which confirmed the enzyme liquefaction enhanced extractable fraction of carotenoids. This observation is probably due to cell wall weakening leading to the depolymerization of pectin chains through polygalacturonase and pectin-lyase activities (Çinar, 2005). It favored cell breakdown during the pressing and so allowed a better release of carotenoids. This higher recovery of phytochemicals using enzymes is consistent with various other studies (Acosta et al., 2014; Carneiro et al., 2002; Çinar, 2005; Puri et al., 2012).
Both these extracts then underwent microfiltration up to a VRR of about 20 using high transmembrane pressure (Figure 4). First, repetitions showed that the process was repeatable with a difference of permeate flux for the same operating conditions of around 10%, which is similar to the repeatability usually achieved using a crossflow microfiltration pilot unit at laboratory scale. Second, the interest of the liquefaction as a pre-treatment is clearly demonstrated from the point of view of process performance. Indeed, combining enzyme liquefaction with physical treatment resulted in the permeate flux being multiplied by 1.5. For the extract with enzyme liquefaction, after a first drop of up to VRR=3, the permeate flux stopped decreasing and remained at around 140 L·h⁻¹·m⁻² up to VRR=19. Therefore, the extraction procedure resulted in a multiphasic solution being obtained, in which high fouling compounds were removed. This positive effect of enzyme liquefaction on the filterability has already been well illustrated in many cases with fruit juices (Domingues et al., 2014; Machado et al., 2012; Pinelo et al., 2010; Rai et al., 2007). It is not only related to the viscosity decrease of the product due to depolymerization and demethylation of the soluble pectic compounds, but probably also to changes of the fouling power of the insoluble fraction (Dahdouh et al., 2016; Vaillant et al., 2008). These insoluble solids mainly consist of tissue and cell fragments that can be greatly modified by pectinolytic and cellulolytic enzymes through the deconstruction of cell walls. Therefore, the interest of the enzymatic treatment was twofold: it led not only to an increase in the carotenoid extraction yield but also to a significant improvement in the performance of the subsequent operation of separation. Because the chosen enzymes were classical adjuvants commonly used in fruit juice industry, they were not purified and consequently were not very expensive. So as often in fruit juice processing, the additional cost of using enzymes would probably be justified by the reduction in investment (membrane area needed) and operating costs (time, energy).
These first trials also confirmed that in all the cases, with or without enzyme liquefaction, carotenoids and SIS were completely retained by the membrane (neither carotenoid nor SIS were found in the permeate, $R = 1$) and that there was no solute retention (same TSS content in permeate and retentate, $R = 0$).

### 3.3. Purification by diafiltration

To improve the purity of the carotenoids, a diafiltration step was added to the concentration process. Diafiltration is simple to carry out, but for industrial application a compromise between permeate flux and water consumption has to be found (Fikar et al., 2010). Indeed, for a targeted purification rate, if the diafiltration is implemented at the beginning of the concentration step, i.e. at low VRR, the permeate flux will be at a very good level but the volume of water needed will be substantial. On the contrary, the higher the VRR chosen for diafiltration, the worse the flux might be, but at the same time, the amount of water needed will be lower. In the case of cashew apple extract, because permeate fluxes were maintained at a high value at high VRR, it was clear that priority had to be given to water savings. For that reason, we chose to conduct diafiltration at the end of the concentration phase. When the retentate volume reached the minimal operational volume in the system, and therefore targeted VRR was achieved, cashew apple extract was replaced by water to feed the device and diafiltration began.

Figure 5 represents the evolution of DVR, VRR, TSS and $J_p$ as a function of time during the process that included diafiltration. The first part of the filtration referred to a regular VRR increase of up to 6-7. During this phase, the volume of retentate was maintained constant by compensating the volume of permeate removed through feeding the system with the same volume of cashew apple extract. Afterwards, a rapid increase of VRR was observed as the feed was stopped in a second phase and thus the volume of the retentate circulating in the concentration loop rapidly decreased. Once the VRR setpoint was reached, diafiltration was
initiated compensating the volume of extracted permeate by the same volume of water and so DVR increased. It is worth noting that after the initial drop, permeate fluxes remained almost constant throughout the concentration and diafiltration steps. Only a slight increase was observed during the diafiltration step, due to the diminution of viscosity caused by dilution with water. In addition, the values obtained were very similar to those obtained previously. From around 230 L·h\(^{-1}\)·m\(^{-2}\) at the beginning, they stabilized at VRR=19 at about 130 L·h\(^{-1}\)·m\(^{-2}\) and 75 L·h\(^{-1}\)·m\(^{-2}\) for enzyme liquefied and untreated extracts respectively. By using enzymes, the time needed to reach VRR=19 was 30% shorter and the time needed to reach a DVR of 3.5, was reduced by 40%. As expected, TSS decreased exponentially in the system as soon as the diafiltration step started. This also implied that the system could be considered as being very similar to a perfectly stirred reactor. Obviously, the time needed to reach a TSS below 5 g·kg\(^{-1}\) was much shorter for the enzyme treated extract because of the greater permeate flux.

Characterization results highlighted that both concentrates exhibited close values considering biochemical analyses (Table 2). TSS decreased considerably following diafiltration. Between 92-96% of solutes were removed from the retentate. While for the insoluble fraction (SIS), it was concentrated 18 to19-fold in accordance with the final VRR. Total carotenoid content was logically higher for enzyme-treated retentate because of the difference between initial contents and of the higher VRR reached. The ratio between the concentration factor CF and VRR was about 1.04 and 1.01 with and without enzymes respectively. Therefore, concentration factors for carotenoids were almost equal to the VRR with and without enzyme liquefaction after diafiltration, as expected. This result showed that there was no loss in carotenoids during processing. It is concomitant with Polidori et al. (2018) who demonstrated similar carotenoid behavior during orange juice microfiltration, using a 0.2 µm membrane with a CF/VRR ratio of 1.06 with or without diafiltration. Chaparro et al. (2016) and Oliveira...
et al. (2016), who studied lycopene concentration from watermelon juice, found a ratio of between 1.00 and 1.06, confirming complete retention of carotenoids. For cashew apple fiber extracts, with or without enzyme liquefaction, the process resulted in a significant improvement of the purity of carotenoids with respect to the total dry matter reaching a purification factor of around 5.

### 3.4. Modelling

From the initial characteristics of the extract (TSS$_0$, SIS$_0$, C$_0$) and assuming that SIS/carotenoid (insoluble fraction) and the TSS (soluble fraction) retentions were respectively equal to 1 and zero, theoretical final values for all characteristics could be evaluated combining equations 4 to 8 for the targeted VRR and DVR values. In all the cases, the predicted compositions of the final retentate were very close to the experimental values (Table 2). A slight deviation can be noticed for TSS, probably because the hypothesis of a perfectly stirred reactor was not entirely true. Nevertheless, these results validated the model with its assumptions. Thanks to a simple set of calculations, it is thus possible to predict the quality of the extract or to simulate situations without carrying out extensive experiments. Therefore, the question of purification rate can be put forward in order to better optimize the diafiltration step. At VRR of 18-19 with DVR of 3-4, the reached purification rates $\alpha$ were close to the maximum, over 0.99. Obviously, the maximum purity that can be achieved with the process is indeed reached. However, through calculation, we showed that a DVR of 1.5 (that corresponds to a final TSS of 11-12 g·kg$^{-1}$) would be sufficient to reach a $\alpha$ of 0.95, which is already a very interesting value. Therefore, the maximum purity that is achievable can be reasonably attained at lower DVR (1.5 instead of 4), allowing water consumption to be more than halved, to reduce processing time by around 20% and to decrease the production of permeate, considered here as an effluent to be treated, by 10%.
This model can be easily generalized for all the VRR/DVR combinations. For example, it can be used to generate charts that link DVR and \( \alpha \) as a function of the chosen concentration factor (Figures 6 and 7). These couples of figures are presented for two different TSS\(_0\)/SIS\(_0\) ratios. The first one corresponds to cashew apple fiber extract with a ratio of 4, and the second one corresponds to a classical fruit juice with a ratio of 33. These charts could be useful to quickly determine the DVR to be used for given concentration level and purification rate. We can notice in the equation set how important the TSS\(_0\)/SIS\(_0\) rate is. The higher this quotient and the lower the concentration factor to be reached, the higher the DVR needed to obtain a targeted purification level. Applied to our extract that had a TSS\(_0\)/SIS\(_0\) value of 4 plotted in figure 6 or to a citrus fruit juice with a TSS\(_0\)/SIS\(_0\) ratio of 33 for instance illustrated in figure 7 (Polidori et al., 2018), the obtained curve pattern is completely different.

For industrial applications a compromise should often be found between permeate flux, concentration factor, purification rate and water consumption. In this respect, the tested model is an interesting tool that should help for the guidance of the process considering the functional potential of the carotenoid concentrate and, the economic cost of the operation.

4. Conclusion

The aim of this work was to evaluate crossflow microfiltration in order to produce a concentrate that is enriched and purified in carotenoids from the extract of a cashew apple by-product. The process coupled with enzyme liquefaction and using a transmembrane pressure around 3 bar, allowed the carotenoid content to be multiplied by up to 19 while keeping the permeate flux above 100 L·h\(^{-1}\)·m\(^{-2}\). In the case of cashew apple fiber extract, this step complies with the reduction of the energy costs which might render the expense of purchasing the enzyme negligible when compared to the increase in volume production. Adding a diafiltration step led to a 5-fold purification of the carotenoids. The final concentrate contained between 0.2 and 0.3 g·kg\(^{-1}\) of carotenoids in dry matter. A simple model was
validated that predicted carotenoid concentration and purity according to the filtration conditions, that is to say the VRR and DVR. This model can be used as a decision aid tool for piloting the process in relation to quality, economic and environmental considerations. This study also showed that cashew apples fibers, that are a low-cost and abundant by-product from the fruit juice industry, especially in Brazil, could be easily treated in order to obtain interesting carotenoid concentrates through the described process. The final extract can be directly used as a natural food coloring and provides an important added value. So, crossflow microfiltration can find its place in sustainable development for the cashew apple processing chain. However, to consider any industrial application, further studies must be conducted on a larger scale, with higher membrane area in order to validate the robustness of the process. Moreover, other aspects such as environmental impact and economic criteria (investment and operation costs) have to be considered in order to demonstrate the interest of an industrial line establishment and scale up, according to the targeted production capacity, equipment availability and local constraints. Eventually, the compositional characterization of the extract has to be completed in order to better evaluate its potential but also to better understand the impact of the process on its composition. The stability of the extract during storage should be investigated in order to determine its shelf life as well.

Acknowledgements & Funding

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REFERENCES


Pinto De Abreu, F., Dornier, M., Dionisio, A. P., Carail, M., Caris-Veyrat, C., & Dhuique-Mayer, C.


Table 1: Composition of the initial cashew apple extracts obtained by pressing, coupled with or without enzyme liquefaction, and used as raw materials for crossflow microfiltration experiments (average value and standard deviation evaluated with 3 repetitions). TSS: total soluble solids; SIS: suspended insoluble solids; DM: dry matter; C: carotenoid content; p: carotenoid purity.

<table>
<thead>
<tr>
<th></th>
<th>TSS (g·kg(^{-1}))</th>
<th>SIS (g·kg(^{-1}))</th>
<th>DM (g·kg(^{-1}))</th>
<th>C (mg·kg(^{-1}))</th>
<th>p (mg·kg(^{-1}))</th>
</tr>
</thead>
<tbody>
<tr>
<td>Without enzyme</td>
<td>52 (1)</td>
<td>12.7 (0.1)</td>
<td>64.7 (1.1)</td>
<td>2.9 (0.1)</td>
<td>45 (2)</td>
</tr>
<tr>
<td>With enzyme</td>
<td>52 (1)</td>
<td>13.0 (0.2)</td>
<td>65.0 (1.2)</td>
<td>3.8 (0.1)</td>
<td>58 (3)</td>
</tr>
</tbody>
</table>

Table 2: Experimental (Exp.) and calculated (Calc.) composition of the concentrates obtained by microfiltration with and without enzyme liquefaction (average value and standard deviation evaluated with 3 repetitions). VRR: volume reduction ratio; DVR: diavolume ratio; TSS: total soluble solids; SIS: suspended insoluble solids; DM: dry matter; C: carotenoid content; p: carotenoid purity.

<table>
<thead>
<tr>
<th>Initial extract</th>
<th>VRR</th>
<th>DVR</th>
<th>TSS (g·kg(^{-1}))</th>
<th>SIS (g·kg(^{-1}))</th>
<th>DM (g·kg(^{-1}))</th>
<th>C (mg·kg(^{-1}))</th>
<th>p (mg·kg(^{-1}))</th>
<th>PF</th>
</tr>
</thead>
<tbody>
<tr>
<td>Without enzyme</td>
<td>17.8</td>
<td>3.81</td>
<td>Exp. 4 (1)</td>
<td>227.0 (0.6)</td>
<td>231.0 (1.6)</td>
<td>54 (2)</td>
<td>234 (10)</td>
<td>5.2 (0.5)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Calc. 1</td>
<td>226.1</td>
<td>227.1</td>
<td>52</td>
<td>228</td>
<td>5.1</td>
</tr>
<tr>
<td>With enzyme</td>
<td>19.1</td>
<td>3.13</td>
<td>Exp. 2 (1)</td>
<td>248.4 (1.5)</td>
<td>250.4 (2.5)</td>
<td>73</td>
<td>292</td>
<td>5.0</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Calc. 1</td>
<td>248.3</td>
<td>249.3</td>
<td>73</td>
<td>290</td>
<td>5.0</td>
</tr>
</tbody>
</table>

content; p: carotenoid purity.
Figure 1: Diagram of the microfiltration device and membrane characteristics

<table>
<thead>
<tr>
<th>Membrane Type</th>
<th>Membralox T1-70 Fall-Eskia</th>
</tr>
</thead>
<tbody>
<tr>
<td>Material</td>
<td>α-4Al2O3</td>
</tr>
<tr>
<td>Pore size diameter (μm)</td>
<td>0.2</td>
</tr>
<tr>
<td>Channel diameter (mm)</td>
<td>7</td>
</tr>
<tr>
<td>Length (mm)</td>
<td>250</td>
</tr>
<tr>
<td>Surface Area (m²)</td>
<td>0.005</td>
</tr>
</tbody>
</table>
Figure 2: Permeate flux ($J_p$) vs. volume reduction ratio (VRR) during the concentration step by crossflow microfiltration of the cashew apple fiber extract without enzyme liquefaction at 5 transmembrane pressures (TmP).
Figure 3: Average permeate flux ($J_p$) vs. transmembrane pressure (TmP) for different intervals of volume reduction ratio (VRR) during the concentration step by microfiltration of the cashew apple fiber extract without enzyme liquefaction.
Figure 4: Permeate flux ($J_p$) vs. volume reduction ratio (VRR) during the concentration by microfiltration of cashew apple extracts with and without enzyme liquefaction ($TmP = 3.2$ bar).
Figure 5: Evolution of A- volume reduction ratio (VRR) and diavolume ratio (DVR), and B- permeate flux ($J_p$) and total soluble solids in the concentrate (TSS) versus time during the concentration and the purification by microfiltration and diafiltration of the cashew apple extracts with and without enzyme liquefaction ($TmP = 3.2$ bar).
Figure 6: Predicted charts from the model, plotting the purification rate of carotenoids ($\alpha$) that is achievable for different diavolume ratios (DVR) and the DVR that is required to reach different $\alpha$, as a function of the concentration factor (CF), for a cashew apple fiber extract with an initial total soluble and suspended insoluble solids ratio $\text{TSS}_0/\text{SIS}_0$ of 4.
Figure 7: Predicted charts from the model, plotting the purification rate of carotenoids ($\alpha$) that is achievable for different diavolume ratios (DVR) and the DVR that is required to reach different $\alpha$, as a function of the concentration factor (CF), for a citrus juice with an initial total soluble and suspended insoluble solids ratio $TSS_0/SIS_0$ of 33.