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Screw Press Extraction and Ultrafiltration of Flavonoids from Kalanchoe Leaves and Stems

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Introduction

In horticulture potted flowering kalanchoe plants and cut flowers are a major product produced and handled world-wide. This production has a great biochain and circular economic potential, as the waste leaves and cut stems from this production are rich in alkaloids, flavonoids, steroids and terpenes, some of which have potential as anti-inflammatories, anti-microbials and anti-allergens, which could find use in medicine and cosmetics.
Kalanchoe has previously been part of traditional natural medicine in the Americas and West Africa (Milad et al, 2014). Previous studies have shown (Rosli et al, 2012), that flavonoids and phenolic compounds can be extracted in aqueous solutions from Kalanchoe Pinnata. Further, flavonoids can be separated using membrane processes such as ultrafiltration (Milani et al, 2015) and nanofiltration (Dzhonova-Atanasova et al, 2018). In the present study these methods were used to process flavonoids from kalanchoe leaves and stems. The aim was to produce a liquid fraction rich in flavonoids, which through further purification could be used as anti-microbials in the cosmetic industry.

Main product:
Cut flowers

Waste stream:
Leaves and stems

Valorized extract:
Anti-microbials

Exhaustively extracted
leaves and stems:
Biomass for biogas

Process Setup

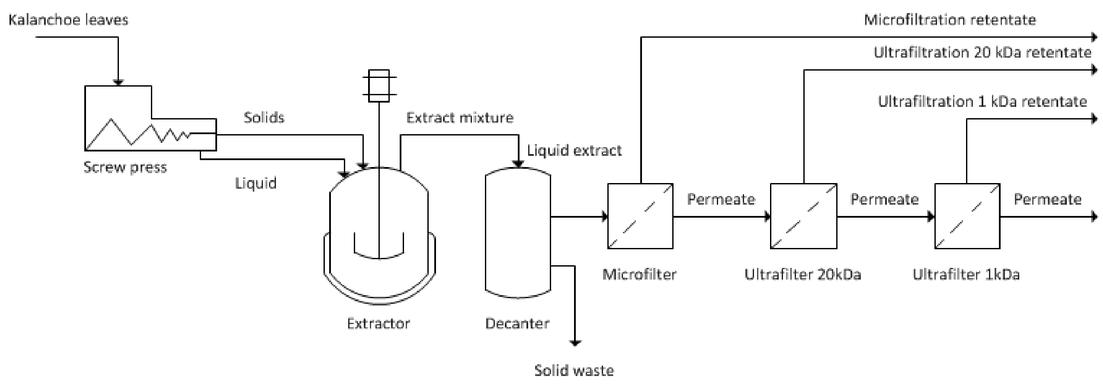

Figure 1. Membranes used for the separation of plant polymers, flavonoids and sugars

The experimental setup is shown in figure 1. The membranes used are shown in table 1.
The leaves and stems are first shredded and mashed into a pulp using a screw press. This is followed by extraction in water at 20 °C. After extraction the larger solids are removed by decantation. The liquid overflow from the decantation still contain fine particulates which are removed by microfiltration. Larger polymer such as tannins, proteins and polysaccharides are then removed by ultrafiltration at 20 kDa. This should allow the flavonoids to pass in the permeate. The final step is to concentrate the flavonoids while removing sugars and amino acids.

Table 1. Membranes used for the separation of plant polymers, isoflavonoids and sugars

Membrane	Producer and Material	Pore size/Molecular Weight Cut Off
Microfiltration MD 020 TP 2N	Microdyn, Polypropylene	0.2 µm
Ultrafiltration GR60PP	Alfa Laval, Polyetehrsulphone/Polysulphone	20 kDa
Ultrafiltration ETNA01PP	Alfa Laval, Composite fluoropolymer	1 kDA

Results

The flux behavior during microfiltration and ultrafiltration is shown in figures 2, 3 and 4.
As seen from figure 2, the flux shows the typical behavior due to solid fouling of the membrane surface during microfiltration: A fast decline in flux from 198 L· m^{-2} ·h^{-1} to 100 L· m^{-2} ·h^{-1} as an initial fouling cake layer builds up, followed by a slower flux decline as minor solids adsorb to the cake layer and inside the membrane pores. At a permeate flux of 68 L· m^{-2} ·h^{-1} the membrane was cleaned. As seen, the original flux could not be reestablished, most likely due to particulates adsorbed inside the membrane pores.
During processing a color change of the microfiltration feed from green towards brown was observed for some of the extraction batches. This change in feed composition did not influence the microfiltration experiments, but it did influence the ultrafiltration flux, as seen in figure 3. The browning of green plant juice is either caused by polyphenoloxidase enzymes that catalyze reactions of phenolic compounds producing quinones, which then polymerize giving rise to melanins, or by Maillard polymerization reactions producing brown pigments. In both cases this leads to brown polymer pigments. The brown feed leads to a lower flux, indicating a much denser fouling layer. This is supported by the fact that the flavonoid retention for the green feed increases slightly from 40% to 44%, while for the brown feed the retention increases from 19% to 83%. The average molecular weight for the flavonoids vary between 282 and 510 Da. Flavonoids should easily permeate through the 20 kDa membrane. Since the flavonoids in both cases are retained by the 20 kDa membrane, the conclusion is that a dense fouling layer have formed. Such dense layers are usually caused by gel layer formation as polymers start to condense close to the membrane surface. The permeate from the 20 kDa ultrafiltration was processed using a 1 kDa membrane. As seen from figure 4 the flux immediately dropped from 130 to 90 L· m^{-2} ·h^{-1} followed by a more gradual decline over five hours to 43 L· m^{-2} ·h^{-1}, after which time the membrane was cleaned. The flux behavior indicates a fast built-up of a fouling layer caused by concentration polarization and a more slow flux decline, as molecules adsorb to this fouling layer and the membrane pores. For the 1 kDa filtration the fouling layer was less dense than for the 20 kDa filtration, as the flavonoid retention was only between 22 and 42%.

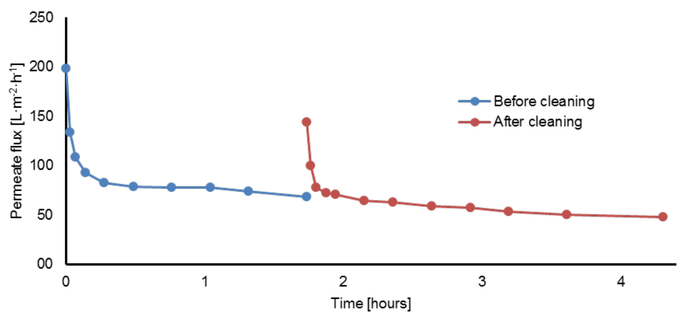

Figure 2. Permeate flux as a function of processing time during microfiltration

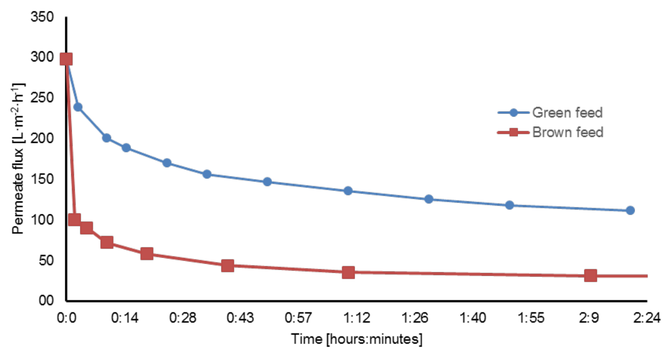

Figure 3. Permeate flux as a function of processing time during ultrafiltration with a 20 kDa membrane.

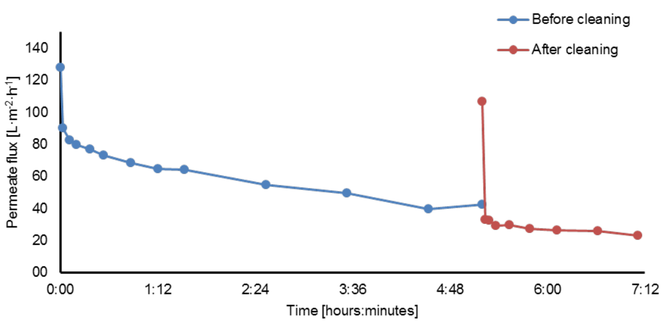

Figure 4. Permeate flux as a function of processing time during ultrafiltration with a 1 kDa membrane.

Conclusions

The combination of screw press extraction, microfiltration and ultrafiltration can produce an aqueous liquid flavonoid extract. However, the membrane separation sequence is not optimal as the fouling layer built-up does not optimally support the separation and concentration of a flavonoid fraction low in polymers, sugars and salts. A possible solution to this problem could be to combine the screw press treatment with an enzyme membrane extraction process using ceramic membranes, as demonstrated by Roda-Serrat et al (2018) for cherry extracts. This seems to produce both a pure flavonoid concentrate and a more stable fouling situation.

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