Screw press extraction and ultrafiltration of flavonoids from kalanchoe leaves and stems

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

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Kalanchoe leaves and stems contain flavonoids known to have anti-inflammatory, anti-microbial and anti-allergenic properties of interest for use in cosmetics and pharmaceuticals. The present study investigates the aqueous extraction of flavonoids from the leaves and stems of kalanchoe hybrids. The extraction process consisted of screw pressing followed by aqueous batch extraction, microfiltration using tubular 0.2 μm polypropylene membranes, 20 kDa polyethersulfone flat sheet membranes and 1 kDa composite fluoropolymer membranes.
The experiments showed that the screw press efficiently separated the kalanchoe leaves and stems into a solid fraction with a dry matter content of 14 % and a liquid fraction with a dry matter content of 2.4 %. Approximately two thirds of the flavonoids were in the liquid phase after the initial pressing. The batch extraction time was found to be 3 hours to achieve maximum flavonoid concentration. The combination of microfiltration and ultrafiltration could produce a liquid fraction rich in flavonoids. However, the proposed combination of membranes and cleaning procedures are not optimal and needs further development.

1. Introduction

In horticulture as in all industry optimizing the economic potential of a production by valorization of waste streams are important. From a biochain and a circular economy perspective, each waste stream is used to its economic optimum and the recycling economy of the available resources is optimized. This increases the revenue per unit mass and decreases the environmental impact by reducing the amount that must be handled as waste. In horticulture potted flowering plants and cut flowers are a major product produced and handled world-wide. This production has a great biochain and circular potential, as the waste leaves and cut stems are still rich in high-value chemicals and the residual biomass can be used for energy production.

Kalanchoe has previously been part of traditional natural medicine in the Americas and West Africa (Milad et al, 2014). Today kalanchoe is produced world-wide in green houses and used as both potted flowering plants and in cut flower arrangements. The leaves and cuts from this production still contain alkaloids, flavonoids, steroids and terpenes, some of which might have potential as anti-inflammatories, anti-microbials and anti-allergens, which could find use in medicine and cosmetics.

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.

2. Materials and Methods

2.1 Materials and Analytical Procedure

Fresh leaves and stems from kalanchoe hybrids were kindly delivered from Queen® (Knud Jepsen A/S, Denmark). Dry matter and water content were determined by placing a sample in an aluminum tray in an oven at 105 °C for 24 hours, weighing the sample before and after drying. The flavonoid content was measured using a standard Ultimax 3000 HPLC Dionex (ThermoFisher Scientific, USA), equipped with a Luna, 5 µm, C18, 150 x 4.6 mm column. The analysis method used two solvents. Solvent A was ultrapure water (ELGA PURELAB) with 0.1 volume-% of formic acid (98%, VWR Chemicals), and solvent B acetonitrile (min. 99.95% super gradient, VWR Chemicals) with 0.1 volume-% of formic acid (98%, VWR Chemicals). The total analysis time was 45 minutes with a flow of 0.8 mL·min^{-1}. The gradient is shown in table 1. The temperature in the autosampler was kept constant at 5 °C to minimize the degradation of the sample during analysis. The column oven was set to 30 °C. A UV detector wavelength at 254 nm was used for quantification and the total flavonoids content was calculated relative to a quercetin standard (>95%, Sigma-Aldrich). The HPLC samples were filtered through a 0.22 µm syringe filter to remove macromolecules that could potentially damage the column.

Table 1: HPLC gradient method

Solvent\Time [min]	0	1	20	25	30	35	40	41	45
A [%]	85	85	75	75	65	1	1	85	85
B [%]	15	15	25	25	35	99	99	15	15

2.2 Experimental Procedure

The overall process flow is shown in figure 1. Twenty kilogram of fresh Kalanchoe leaves were first mashed in a screw press (Spiral Juicer from Orange Mechanical Equipment, China) with a 87.5 mm screw extruder and a screen with hole diameters of 0.6 mm. The mashed leaves and press liquor were then mixed and extracted with demineralized water under stirring in a 150 L stainless steel tank equipped with a marine propeller, the water to plant ratio being 5:1 based on weight. The extraction temperature was 20 °C and the extraction time was two hours, after which time the flavonoid concentration in the liquid phase became constant. The extract mixture was transferred to 20 L polypropylene tanks and allowed to settle for 24 hours at 5 °C to reduce degradation of the flavonoids. The liquid phase was then decanted for further processing.
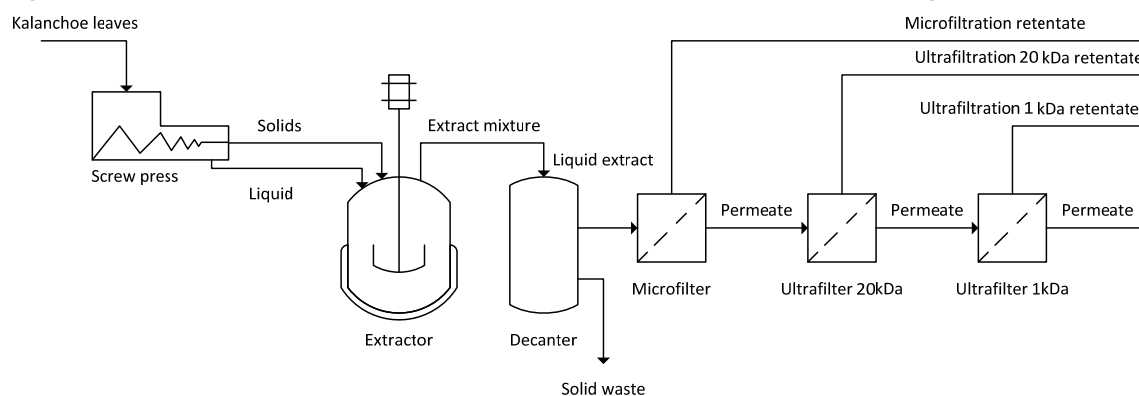

Figure 1: Overall process flow

To avoid microbial degradation during the further flavonoid separation process and to remove solid residues, the liquid was microfiltered using four tubular 0.2 µm polypropylene membranes (MD 020 TP 2N, Microdyn Nadir, Germany) in parallel with a total filtration area of 0.144 m^2. The microfiltration was done at 30 °C and 1.6 bar average transmembrane pressure. The cross flow velocity was 1.5 m/s. The hydrophobic membranes were activated using 70% ethanol followed by flushing with demineralized water. The setup is illustrated in Figure 2. The feed temperature was controlled by recycling hot water from tank T-104 to tank T-101. Under normal operation the feed was pumped from T-101, to the membrane manifold. By opening or closing the valves V-102/4/8 or V-103/5/9, it was possible to change the number of membranes (M-101/2) in parallel from four to eight. During filtration valve V-106 was open to collect the permeate in tank T-103. The retentate pressure was controlled by valve V-111. TR101/2 and PR101/2 measured temperature and pressure, respectively.The permeate flow was found by weighing. The retentate flow was measured by flowmeter FI-101.
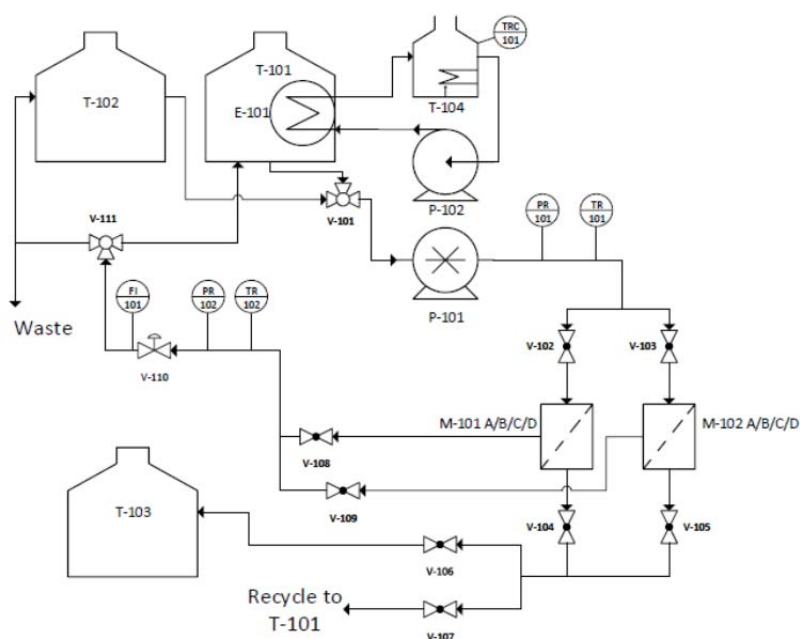
Figure 2: PI diagram of the microfiltration setup.

Membrane cleaning was done by flushing the system with demineralized water followed by recycling of a pH 10 sodium hydroxide solution for 30 minutes, and finally cleaning with demineralized water until a neutral pH was reached. The permeate from the microfiltration will contain polysaccharides, protein residue, flavonoids and sugars. To remove the larger polymers a flat sheet polyethersulfone 20 kDa ultrafiltration membrane (GR60PP from Alfa Laval, Denmark) was used. The permeate from this ultrafiltration was send to a 1 kDa flat sheet composite fluoropolymer ultrafiltration membrane (ETNA01PP, Alfa Laval, Denmark), which was expected to allow sugars and salts to pass, but at the same time show a high rejection of the flavonoids. The total filtration area for both of these ultrafiltration steps were 0.072 m^2, the average transmembrane pressure was 5.8 bars, and the processing temperature was 30 °C. Both ultrafiltration steps used the process setup illustrated in Figure 3.
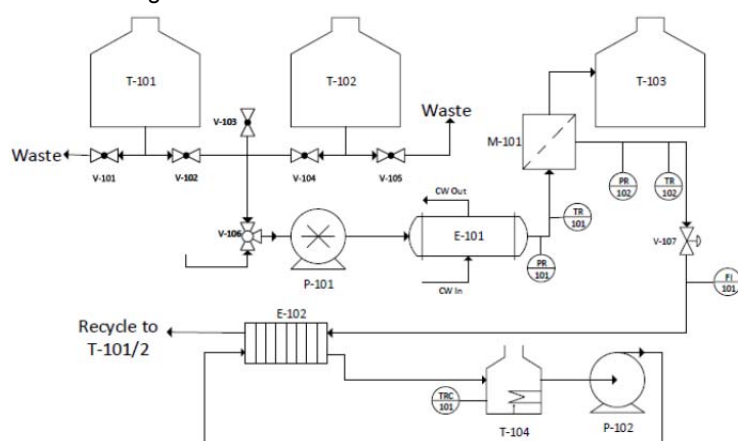

Figure 3: PI diagram of the ultrafiltration setup using a M20 LabStak (Alfa Laval, Denmark).

The feed temperature was controlled by recycling water from tank T-104 through heat exchanger E-102. Under normal operation the feed was pumped from T-101 to the M20 LabStak, which includes heat exchanger E-101 and the membranes (M-101). Valve V-107 controlled the retentate pressure. TR101/2 and PR101/2 measured temperature and pressure, respectively. The permeate flow was found by weighing. The retentate flow was measured by flowmeter FI-101. The cleaning procedure was identical to that used for microfiltration, except that the sodium hydroxide cleaning was followed by a pH 3 citric acid cleaning for approximately 30 minutes.

3. Results and Discussion

The fresh kalanchoe leaves and stems had a dry matter content of 4.4 weight-% ± 0.5 weight-%. Eighty-seven percent by weight plus/minus one percent by weight of the feed ended up in the liquid fraction after the screw press. The dry matter concentration in the liquid fraction from the screw press was 2.4 weight-% ± 0.1 weight-%, while the dry matter content in the solid fraction was 14 weight-% ± 1 weight-%. Further analysis of the samples showed that roughly two thirds of the flavonoids were already in the liquid phase prior to extraction. This is not surprising as the flavonoids are mainly present in their glycosylated form, making them highly water soluble. The following extraction step is thus more to be looked upon as a leaching step, removing flavonoids from the solid phase by dilution of the liquid trapped between the solid particulates. This is supported by the relative short extraction time as seen in figure 4.
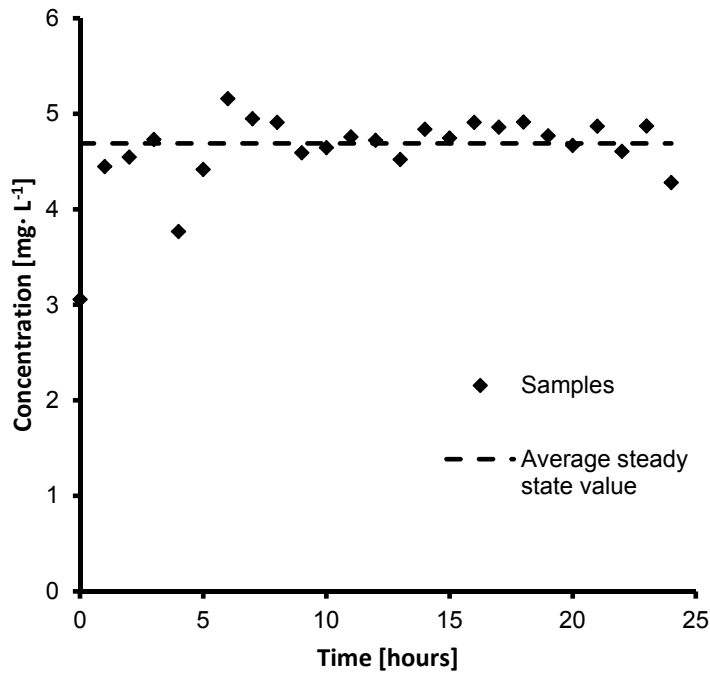

Figure 4: Flavonoid concentration increase during extraction as a function of concentration time.
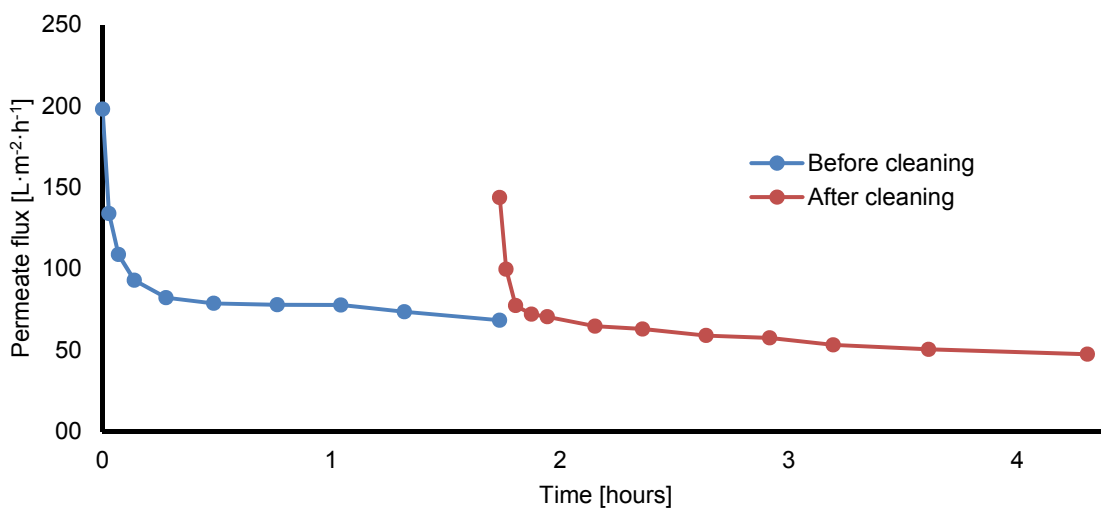

Figure 5: Permeate flux as a function of processing time during microfiltration

The liquid fraction decanted from the extraction mixture was clarified by microfiltration. As seen from figure 5, the flux shows the typical behavior due to solid fouling of the membrane surface during microfiltration: First a fast decline in permeate 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 (Baker, 2012). 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 processed. While an influence of this change in feed composition could not be seen in the microfiltration experiments, it did influence the behavior of the ultrafiltration experiments, as seen in figure 6. 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 (Queiroz et al, 2008), or by Maillard polymerization reactions producing brown pigments (Burdurlu & Karandiz, 2003). 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, depending on whether they are in their glycosylated form or not. Flavonoids should easily permeate through the 20kDa membrane. Since the flavonoids in both cases are retained by the 20 kDa membrane, the conclusion is that a dense fouling layer must have formed. Such dense layers are usually caused by gel layer formation, when polymers start to condense close to the membrane surface because of concentration polarization (Baker, 2012).
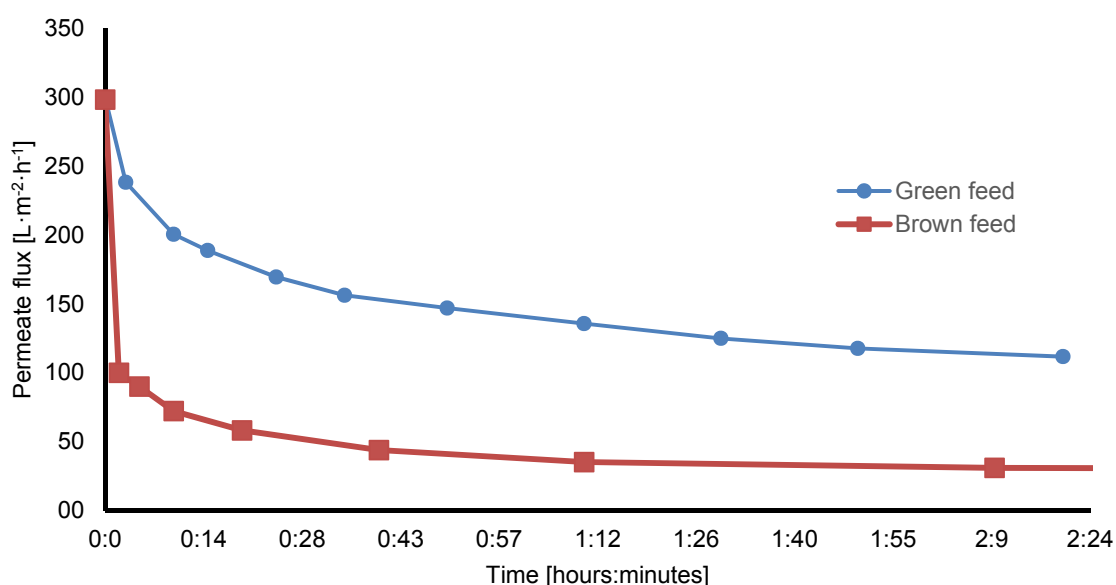

Figure 6: Permeate flux as a function of processing time during ultrafiltration with 20 kDa membrane.

The permeate from the 20 kDa ultrafiltration was processed using the 1 kDa membrane. As seen from figure 7 the permeate 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. This restored the membrane flux to 107 L·m^{-2}·h^{-1}. Again the flux immediately declined to 33 L·m^{-2}·h^{-1} followed by a slow decline over the next two hours to a flux of 23 L·m^{-2}·h^{-1}. This 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%. This is perhaps not surprising, as most of the polymers able to form a dense fouling layer must have been removed by the 20 kDa filtration prior to the 1 kDa filtration. For all filtration processes the applied cleaning procedures are obviously insufficient, as the initial flux cannot be restored. The flux decline behavior indicates that for microfiltration solids adsorb irreversible inside the membrane pores, and that for ultrafiltration the same happens for molecules inside the membrane pores. Further, the flavonoid retention for the 20 kDa membrane is higher than for the 1 kDa membrane. This is of

course not satisfactory, as a flavonoid concentrate free of polymers, sugars and salts is the purpose of the process.
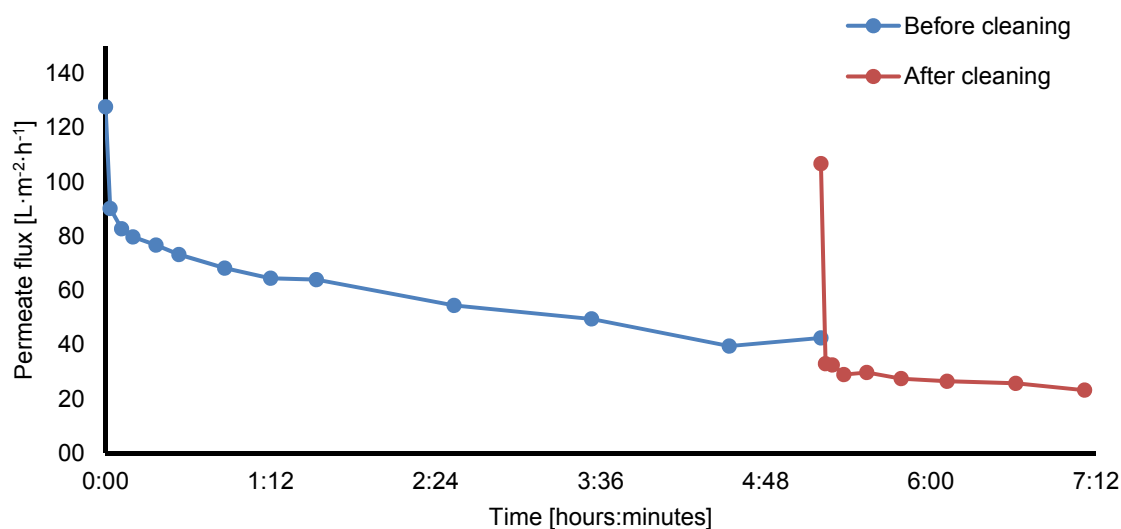

Figure 7: Permeate flux as a function of processing time during ultrafiltration with a 1 kDa membrane.

4. 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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