Concentration of phenolic compounds from apple pomace extracts by nanofiltration at lab and pilot scale with a techno-economic assessment

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Abstract

Apple pomace can be used as resource for the extraction of phenolic compounds with anti-oxidant properties. Pressing of apple in juice and pomace at lab scale in open air (aerobic) and under N_2 atmosphere (anaerobic) showed a recovery of phenolic compounds of 85% in juice and pomace after anaerobic pressing, compared to 43% after aerobic pressing, indicating loss of phenolic compounds by oxidation and the advantage of anaerobic over aerobic pressing. After a membrane screening and concentration test at lab scale, the commercial nanofiltration membrane NFX was selected to concentrate phenolic compounds in an ethanol:water extract of apple pomace. At pilot scale, the concencentration of ten selected phenolic compounds and quinic acid increased from 59.5 mg L^{-1} in the ethanol:water extract to 1256.1 mg L^{-1} in the final retentate, i.e. by a factor 21.1. The volume of the crude extract was reduced by a factor of 28.5 during the filtration, indicating some loss of phenolic compounds during pilot testing due to membrane fouling or oxidation of polyphenols. The pilot concentration test using a spiral-wound membrane module showed good flux and concentration of phenolic compounds, indicating the technical feasibility of membrane technology for efficient concentration of polyphenols in an ethanol:water extraction solvent. Unfortunately, the extraction and concentration process was not economically feasible under the assumptions made.

Practical Applications

The valorization of food waste for the production of high-added value products is an increasingly hot topic. Phytochemicals are present in relatively low concentration in the fruit matrix and concentration in the extraction solvent is necessary to develop an industrially relevant process. In this study, membrane filtration was selected for concentration due to its low energy consumption and mild processing conditions compared to other technologies. Membrane screening and testing at lab and pilot scale with techno-economic assessment can

be used by food and nutraceutical industries to evaluate membrane technology for concentration of phytochemicals extracted from agroindustrial by-products.

Keywords

Phenolic compounds, apple pomace, nanofiltration, pilot scale, techno-economic assessment

Introduction

Food processing by-products are often used as feed or bioenergy source. In general, they still contain useful major compounds like carbohydrates, proteins and fats, and minor compounds like polyphenols, carotenoids or terpenes. These compounds are high-added value valorisation products from food waste. The use of food waste as renewable resource for the production of chemicals, materials and fuels is getting more and more attention (Lin *et al.* 2013; Garcia-Gonzalez *et al.* 2015).

Apple pomace is a left-over residue (25-30% of the total processed apple) after pressing of apple to juice. Worldwide, 3 to 4.2 million tonnes of apple pomace are generated per year (Lin *et al.* 2013). Apple pomace is used for several applications like feed or as substrate for biotechnological applications like production of enzymes, single cell protein, ethanol and organic acids (Schieber *et al.* 2003; Bushan *et al.* 2008; Vendruscolo *et al.* 2008; Wijngaard and Brunton 2010). On an industrial scale, apple pomace is used for the production of pectin. Extraction of phytochemicals from waste products, such as apple pomace, has received much interest in recent years due to the use of natural and low cost sources of phytochemicals for incorporation into foods or beverages (Schieber *et al.* 2003; Wijngaard and Brunton 2010; Harbourne *et al.* 2013). Apple pomace contains many polyphenols including chlorogenic acid, catechins, procyanidins and quercetin glycosides (Harbourne *et al.* 2013). Polyphenols are secondary plant metabolites with anti-oxidant properties. Phenolic compounds can be easily oxidized by exposure to air during the pressing of apple in the juice industry and are lost in a process called enzymatic browning (Van der Sluis *et al.* 2002). Enzymatic browning is the oxidation of polyphenols by polyphenol oxidase, resulting in a brown colour. It has two disadvantages: production of a brown colour which is not attractive for consumers and the decrease in concentration of phenolic compounds in the apple juice (Van der Sluis *et al.* 2002; De Paepe *et al.* 2015a) with decrease in value.

Antioxidants work effectively as disease preventing species and a consensus exists that a diet rich in fruit and vegetables is beneficial for health in preventing coronary heart disease and some forms of cancer (Matés 2013).

Phytochemicals make up less than 10% of the plant matrix (Harjo *et al.* 2004), therefore they need to be extracted to prepare phytochemical rich foods and beverages (Sarmento *et al.* 2008; Harbourne *et al.* 2013). Organic solvents are often used for extraction of phytochemicals. Polyphenols can be extracted with conventional solvents like water, methanol, ethanol, ethyl acetate and acetone or a mixture of these solvents (Sarmento *et al.* 2008). Hasbay Adil *et al.* (2007) used subcritical CO_2 and ethanol for extraction of polyphenols from apple pomace. After optimization with response surface methodology, Wijngaard and Brunton (2010) used 56% ethanol or 65% acetone as food grade solvents for extraction of polyphenols from apple pomace. Since the concentration of polyphenols in fruit and vegetables is relatively low, the polyphenols in the extraction solvent need to be concentrated to obtain a feasible process at industrial scale. Membrane separation technology offers several advantages like low energy consumption and mild operating conditions for separation of thermolabile compounds (Sarmento *et al.* 2008), compared to more traditional separation methods like distillation or adsorption. Nawaz *et al.* (2006) used ultrafiltration membranes to concentrate polyphenols from grape seeds, while Sarmento *et al.* (2008) used polymeric membranes for concentration of polyphenols from cocoa seeds. Saleh *et al.* (2006) used nanofiltration membranes with molecular weight cut-off of 250 and 1000 Da to recover phenolic compounds from apple juice for nutraceutical use.

In this study, the optimized solvent extraction of Wijngaard and Brunton (2010), i.e. ethanol 56% or acetone 65%, was used to recover phenolic compounds from apple pomace, and nanofiltration membranes were subsequently used to concentrate polyphenols in the extraction solvent at both lab and pilot scale. Furthermore, a techno-economic assessment (TEA) was made to study the economic feasibility of the extraction and separation process and to determine the most important process parameters. The TEA exists of four different, integrated steps: (1) market analysis, (2) process flow diagram and mass and energy balance, (3) economic analysis, and (4) uncertainty analysis (Van Dael *et al.* 2015). Taking into account the uncertainty of the data, the last step is crucial in order to provide food waste processors a proper insight in the techno-economic performance of the process. To our knowledge, this is the first report of the concentration of phenolic compounds from apple pomace extracts by nanofiltration at pilot scale with a TEA in scientific literature.

Materials and methods

Plant material

The apple cultivar used for the pressing on lab and pilot scale was 'Golden Delicious' obtained from a local distributor in Belgium. The fresh apples were stored at 4 °C until use.

Mechanical pressing of apples at lab scale

One kg of apples with peels and seeds, but without stalks was cut in eight pieces and mechanically pressed using a screw press into two fractions: apple juice and apple pomace. For the aerobic experiments, the apples were cut and pressed in open air. For the anaerobic experiments, the apples were cut and pressed in an anaerobic glove box filled with N_2 to prevent oxidation of the phenolic compounds.

Mechanical pressing of apple at pilot scale

The pressing of the apples at pilot scale was performed at the Food Pilot of the Institute for Agriculture, Fisheries and Food Research (ILVO) in Melle, Belgium. First, the apples with peels, seeds and stalks (200 kg) were cut with a grinding system (Multicut 1500, Bruckner Liquid Food Tech – VaculIQ, Germany) under N_2 atmosphere to prevent oxidation of polyphenols in the apple juice and pomace, and then pumped to the spiral-filter press (VaculIQ 1000-300, VaculIQ, Germany) as described by De Paepe *et al.* (2015a). During the stabile phase of the pressing (after 30 minutes of pressing), the juice and the pomace were collected during one minute to calculate the juice yield as described by De Paepe *et al.* (2015a). The apple pomace was sampled immediately after leaving the press to prevent oxidation of the polyphenols and vacuum packed in plastic bags (subsamples of 2 kg). The apple pomace and apple juice were immediately frozen at -25 °C and stored at -18 °C until extraction. An overview of the process parameters of the grinding system and the spiral-filter press is shown in Table 1. The juice yield was very high (83.3%), indicating a

production of 83.3 kg apple juice (and 16.7 kg apple pomace) starting from 100 kg fresh apple (if no losses during pressing are taken into account).

Analytical extraction of phenolic compounds

The analytical extraction of phenolic compounds from apple, apple pomace and apple juice was performed on freeze-dried samples. The samples were immediately frozen in liquid nitrogen to avoid enzymatic browning and transferred into a freeze dryer with heated shelves at 25 °C (GAM-MA 1-16 LSC Martin Christ, Germany). After the freeze-drying process, the samples were grounded in a commercial blender (DP705 La Moulinette, Belgium) and stored under N_2 atmosphere in an amber-coloured flask at -18 °C.
The phenolic compounds were further extracted from the freeze-dried samples according to the detailed protocol of De Paepe *et al.* (2013).

Extraction of apple pomace at lab scale

The polyphenols in the apple pomace were extracted in erlenmeyer flasks with the method described by Wijngaard and Brunton (2010). The apple pomace was freeze-dried (as described above) prior to extraction for the membrane screening tests or extracted fresh for the lab concentration tests. Extraction conditions for ethanol 56% were an extraction temperature of 80 °C and 31 min extraction time. Extraction with acetone 65% was performed at 25 °C during 60 min. The solid to liquid ratio was 10 g dry weight (DW) apple pomace L^{-1} solvent. After extraction, the samples were centrifuged for 10 min at 931 g (Beckman Coulter Allegra X-15R, USA) and 10 mL of the supernatant was filtered through 0.22 µm PVDF syringe filters (Pall Gelman Laboratory, UK). The solvents used were analytical grade and purchased from VWR (Belgium). The extracts were stored under N_2 atmosphere at -18 °C.

Extraction of apple pomace at pilot scale

The extraction of the phenolic compounds from the apple pomace at pilot scale was performed at the chemical pilot plant of Agfa-Gevaert NV in Westerlo, Belgium. The pilot plant was chosen for explosion safety due to the extraction with ethanol:water at 80 °C.

Prior to extraction, the frozen apple pomace was defrosted overnight at 4 °C. The extraction was performed in a 1000 L chemical reactor of glass enamel. The reactor was filled with 252 kg ethanol and 198 kg distilled water and heated under N_2 atmosphere up to 75 °C. The apple pomace (26.8 kg) was added to the reactor and extracted (under N_2 atmosphere) at 80 °C during 31 minutes under continuous stirring at 120 rpm with a three-blade impeller. After extraction, the reactor was cooled to 25 °C during 75 minutes. The solid to liquid ratio was 12 g DW apple pomace L^{-1} solvent.

The extraction solvent was separated from the apple pomace by filtration using a bag filter with pore size 100 µm, followed by a candle filtration (Roki PEH pore size 2 µm, ROKI Techno, Japan) at a pressure of 0.3 bar. The filtration was performed under N_2 atmosphere. The candle filtration was performed with one candle filter for every 30 L of extraction solvent. The filtrate (380 kg) was stored in two 200 L steel drums, flushed with N_2, and stored at 4 °C.

Analysis of phenolic compounds

Identification and quantification of the selected phenolic compounds were performed via an Ultra High Performance Liquid Chromatography-Mass Spectroscopy (UHPLC-MS) method, as described in detail by De Paepe *et al.* (2013). An analytical standard was used for the calibration of each individual phenolic compound.

Membrane screening

The membrane screening was carried out on a high pressure bench-top cross-flow filtration unit equipped with a temperature controlled feed vessel (1000 mL), a circulation pump and a membrane test cell. The transmembrane pressure was generated by N_2 gas. A circular, flat test cell (Amafilter, the Netherlands) with an active surface area of 0.0044 m^2 was used. The membrane coupons were sealed with Kalrez® o-rings. MEFIAS software was used for process monitoring. An overview of the used commercial nanofiltration membranes with nominal molecular weight cut-off (MWCO) is shown in Table 2. Two types of nanofiltration membranes were selected, i.e. standard polyamide-based membranes for water filtration, and organic solvent nanofiltration (OSN) membranes (Vandezande *et al.* 2008; Marchetti *et al.* 2014).

The feed solutions (450 mL) were an ethanol:water and an acetone:water extract from apple pomace, prepared by the extraction method at lab scale. The membrane screening was carried out in batch mode at a flow rate of 800 L h^{-1}, corresponding with a cross-flow velocity of approx. 1.7 m s^{-1}, a temperature of 22 ± 1 °C and a trans-membrane pressure of 20 or 30 ± 1 bar. The initial feed after circulation was sampled, as well as the permeate and retentate at steady-state conditions. All samples were stored under N_2 atmosphere at -18°C until analysis.

Concentration test at lab scale

Two laboratory stainless cross-flow filtration units with capacity of 1000-4000 mL (CF1) and 300-1000 mL (CF2) were used. The feed solution (3500 mL) was an ethanol:water extract from apple pomace, prepared by the extraction method at lab scale. The ethanol:water extract was first concentrated on CF1 until approx. 1000 mL and was further concentrated on CF2 until approx. 300 mL. The transmembrane pressure was generated by N_2 gas. A rectangular test cell (PS Prozesstechnik GmbH, Switzerland) with active membrane surface area of 0.01 m² was used for both CF1 and CF2. The commercial flat polyamide-based membrane NFX (Table 2) was used without specific pretreatment. The membrane coupons were sealed with Kalrez® o-rings. MEFIAS software was used for process monitoring of permeate flux. The concentration test was carried out at a flow rate of 800 L h^{-1} (cross-flow velocity of approx. 1.8 m s^{-1}), a temperature of 20 ± 1 °C and a trans-membrane pressure of 20 ± 1 bar. The initial feed after circulation was sampled, as well as the permeate and retentate at regular time points during concentration at increasing volume concentration factor (VCF). All samples were stored under N_2 atmosphere at -18°C until analysis.

Concentration test at pilot scale

The concentration of the phenolic compounds from the ethanol:water extract was performed with a mobile, semi-automatic, cross-flow solvent pilot unit (ATEX design) with a feed tank of 400 L. The concentration test at pilot scale was also conducted with the commercial membrane NFX (Table 2), this time using a 3838 spiral-wound module (3.8 inch diameter, 38 inch length, 31 mil feed spacer) with active surface area of 8.92 m² (Synder Filtration,

USA). The feed volume at pilot scale was 409 L and batch concentration was performed during 8 days, until minimal feed volume (60 L). The membrane module was operated at a feed flow of 6500 Lh^{-1}, corresponding with a cross-flow velocity of approx. 2 m s^{-1}. Further concentration of the final retentate of the pilot scale test was performed at lab scale using test unit CF1 and a rectangular cell (0.01 m^2) with a flat NFX membrane, as described above. The feed volume (4.8 L) for the lab scale test, following pilot scale, was further concentrated during 9 days. The pilot test and further concentration at lab scale were conducted at 20 ± 1 °C and 20 ± 0.25 bar. Samples were taken and stored as described at lab scale.

Membrane flux and retention

The membrane flux J (kg m^{-2} h^{-1}) was determined by weighing the permeate samples and calculated according to

$J = m/At$

with m the weight of the permeate per unit membrane area A and time t. The density of the solvents was used to convert the flux in L m^{-2} h^{-1}.

The retention of component i (R_i) was calculated according to

$R_i = (1-Cp_i/Cr_i)$ x 100%

with Cp_i and Cr_i the concentrations of component i in the permeate and in the retentate, respectively.

Techno-economic assessment (TEA)

For the TEA, an economic lifetime of ten years and a weighted average cost of capital (WACC) of 5.41% was assumed. Based on Eurostat data, a labour cost of 39 euro h^{-1} (cost for Belgium in 2015, http://ec.europa.eu/eurostat/statistics-explained/index.php/Hourly_labour_costs, accessed on July 14, 2016) and an electricity price of 93 euro MWh^{-1} (price for industrial consumers in Belgium with a consumption of 20.000-70.000 MWh $year^{-1}$, year 2015 semester 2, all taxes included, http://ec.europa.eu/eurostat/web/energy/data/database, accessed on July 14, 2016) was chosen. The maintenance costs for the total process were estimated at 2.5% of the investment costs. A water price of 3.7 euro m^{-3} (average price for a company in Flanders,

Belgium in 2012 with a consumption of 1000 m^3 $year^{-1}$, https://www.vmm.be/publicaties/vergelijking-van-de-kostprijs-van-water-afvalwater-hemelwater-voor-de-gebruikers-in-verschillende-europese-landen, accessed on July 14, 2016) and an ethanol price of 0.55 euro L^{-1} (price of January, 2016, http://www.platts.com/price-assessments/agriculture/ethanol, accessed on July 14, 2016) was assumed. The selling price of polyphenols amounts to 28 euro kg^{-1}, based on our market analysis.

Results and Discussion

Fractionation of phenolic compounds between apple pomace and juice

De Paepe *et al.* (2015a) selected ten marker phenolic components for apple fruits: chlorogenic acid, isoquercitrin, hyperin, rutin, avicularin, quercitrin, phlorizin, catechin, epicatechin and procyanidin B2 (Table 3). Quinic acid is a cyclic polyol and not a polyphenol since it does not contain an aromatic ring. It was included in this study since it is an important secondary metabolite in apple fruits with similar structure.

An overview of the concentration of these ten selected phenolic components and quinic acid in apple fruits is shown in Table 4. In total, the apple fruits contained 3.8 mg g^{-1} dry weight (DW) phenolic compounds (without quinic acid). This total concentration is higher compared to De Paepe *et al.* (2015b), who analysed phenolic compounds in 47 apple cultivars. The flesh (without peel) of the same apple cultivar contained 1.4 mg g^{-1} DW, while in our study also the peel of the apple was included in the sample, explaining the higher concentration of phenolic compounds.

The apples were pressed in apple juice and apple pomace under aerobic and anaerobic conditions. The recovery of phenolic compounds compared to the phenolic concentration in the total apple was 85% in juice and pomace after anaerobic pressing, compared to only 43% after aerobic pressing, indicating the advantage of anaerobic pressing over aerobic pressing. Some loss of apple (5-6%) was observed in the press, leading to a decrease in recovery. Furthermore, in intact plant cells, polyphenols and polyphenol oxidase are physically separated in distinct compartments (Renard *et al.* 2001): the polyphenols are present in the vacuoles and the polyphenol oxidase in the chloroplasts (Vela *et al.* 2003).

When cells are ruptured by pressing, the polyphenols come into contact with the polyphenol oxidase with subsequent loss of phenolic compounds by oxidation. Oxidation of polyphenols in apple juice and pomace should be avoided, for example by vacuum-deaeration, gas sparging or ascorbic acid addition as often used in the juice industry (Garcia-Torres *et al.* 2009).

Extraction of polyphenols

The total concentration of the ten marker phenolic compounds was 3.0 mg g^{-1} DW for ethanol:water, compared to about 3.4 mg g^{-1} DW for acetone:water (Table 5). This indicates that acetone:water is a slightly better extraction solvent for polyphenols from apple pomace, compared to ethanol:water. These data are in comparison with Wijngaard and Brunton (2010).

Membrane screening

A set of selected membranes was screened for an ethanol:water and an acetone:water extract. The time-average permeate fluxes at steady-state conditions of the tested membranes are summarized in Table 6.

In general, the selected phenolic compounds and quinic acid are removed well from the ethanol:water and acetone:water extracts (data not shown), with retentions well over 90%, in many cases over 95%. In general, the difference in retentions among the tested membranes is very small. Several studies reported comparable polyphenol retentions of 93 to 100% for comparable commercial polymeric nanofiltration membranes (Sarmento *et al.* 2008; Tylkowski *et al.* 2010; Cissé *et al.* 2011) . The permeate flux of the membranes tested on the ethanol:water extract ranges by a factor two, i.e. 12 L m^{-2} h^{-1} for DuraMem200 to 23 L m^{-2} h^{-1} for NF90, both at 20 bar. As comparison for the NF90 membrane, Machado *et al.* (2013) reported a lower average permeate flux of 7 L m^{-2} h^{-1} for an ethanol 95% extract of pequi, but also at a lower pressure of 8 bar. For the acetone:water extract, the fluxes of NF030306 and NanoPro AS3012 are about one order of magnitude lower than those of DuraMem200 and DuraMem300. The DuraMem200 membrane displays an increased flux at increased operating pressure, which was also observed by others (Cissé *et al.* 2011; Couto *et al.* 2011; Acosta *et al.* 2017) for other nanofiltration membranes. A membrane with high

retention (more than 95%) of phenolic compounds and high permeate flux is essential to obtain a yield-optimized concentration process. Among the tested membranes, NF90 and NFX perform best for the ethanol:water extract, while DuraMem200 (30 bar) is the most suitable membrane for the acetone:water extract. NF270, a similar nanofiltration membrane as NF90 and NFX with MWCO of 150-300 Da, showed the highest potential for concentration of polyphenolic compounds from blackberry juice (Acosta *et al.* 2017). Machado *et al.* (2013) used the NF90 membrane for the concentration of polyphenols from an alcoholic and aqueous extract of pequi, a typical Brazilian fruit. Wijngaard and Brunton (2010) concluded that both ethanol:water and acetone:water are suitable to replace methanol for a food grade and more environmentally friendly extraction of polyphenols from apple pomace. In this study, the ethanol:water extraction method was selected over acetone:water to produce a polyphenol rich extract from apple pomace for further concentration tests after personal communication with several food companies.This selection is in accordance with Machado *et al.* (2013), who used ethanol for polyphenol extraction due to its GRAS (generally-recognized-as-safe) status. Furthermore, the acetone:water extracts need significantly more expensive organic solvent nanofiltration membranes, compared to cheaper regular membranes for water filtration for the ethanol:water extracts. The NFX membrane was selected over the NF90 membrane after communication with membrane suppliers Synder Filtration and Dow Filmtec on the (potential) suitability of their spiral-wound modules (membranes, glues, spacers) for solutions with an ethanol content as high as 56%.

Concentration test at lab scale

A longer term batch concentration test at lab scale was conducted with the NFX membrane to further investigate the feasibility of nanofiltration to concentrate phenolic compounds from an ethanol:water extract of apple pomace. For the ethanol:water extract, the total volume concentration factor (VCF), i.e. the volumetric ratio of the initial feed to the final retentate, was 32.4. Fig. 1 shows the evolution of the permeate flux of the membrane as function of the VCF. As expected, fluxes decrease with increasing VCF, which can be explained by increasing solute concentrations in the boundary layer at the feed side of the membrane. For the ethanol:water extract, the flux decreased from 24.9 to 3.6 L m^{-2} h^{-1} at final VCF. The feed/retentate concentrations of the ten selected phenolic compounds and quinic acid are plotted as function of increasing VCF in Fig. 2 for the ethanol:water extract.

The differences in retention behaviour of individual polyphenol compounds appear to point to differences in concentration, functionality and affinity with the membrane surface, and interactions with macromolecules co-extracted with the polyphenols. A systematic study using model mixtures with increasing complexity would allow to gain more insight into the phenomena underpinning the differences observed. This is however beyond the scope of the present study. The results show an increase of the total phenolic concentration (sum of ten selected phenolic compounds and quinic acid) from 38.7 mg L^{-1} in the feed to 718.8 mg L^{-1} in the final retentate, i.e. by a factor of 18.6 for the ethanol:water extract, compared to a total VCF of 32.4. Both factors are not equal and this can be explained by losses of phenolic compounds upon sampling, oxidation or fouling of the membranes. Membrane fouling during apple juice clarification may be caused by pectins, tannins, proteins, starch, hemicellulose and cellulose (Mondor *et al.* 2000).

For the ethanol:water extract, the average retention of the phenolic compounds was 98-99%, except for quinic acid (96%), catechin (83%) and epicatechin (93%), due to their relatively low molecular weight (192 Da for quinic acid and 290 Da for catechin and epicatechin). The other phenolic compounds have a higher MW in the range 354-611 Da. These observations can be explained by the nominal MWCO of the NFX membrane of 150-300 Da, which is defined as the molecular weight of the solute that is retained for 90% by the membrane (Mustafa *et al.* 2014). Machado *et al.* (2013) showed a 97% retention of total polyphenols from an aqueous extract of pequi, compared to only 15% retention from a 95% ethanol extract using a similar NF90 membrane. The large difference in retention between aqueous and ethanol extract was explained by the hydrophilic nature of the NF90 membrane and this can be affected by hydration/solvation of the pore wall. The effective pore size could be the smallest in water and the largest in ethanol. In our study, 56% ethanol was used and not 95% ethanol, explaining the good retention by the hydrophilic NFX membrane.

Pilot concentration test

The initial feed (409 L) was concentrated on the pilot unit until 60 L, corresponding to a VCF of 6.9, in about 8 days. Afterwards, a subsample (4.82 L) of the obtained concentrate was further concentrated on the lab scale unit CF1 until 1.16 L, corresponding to a VCF of 4.2 (about 9 days of operation). Hence, a total VCF of 28.5 was reached.

Fig. 3 shows the evolution of the permeate flux of the membrane as function of the VCF. As expected, fluxes decrease with increasing VCF, which can be ascribed to increasing solute concentrations at the feed side of the membrane and membrane fouling, as described for the lab concentration tests. During the pilot test, the flux decreased from 6.8 intially to 0.4 L m^{-2} h^{-1} at VCF 6.9. Cissé *et al.* (2011) showed a decrease in flux over time from 40 to about 5 L m^{-2} h^{-1} at a VCF of about 6 for a similar flat-sheet nanofiltration membrane for an aqueous roselle extract at semi-industrial scale. At the start of the lab test with the retentate of the pilot test, the flux increased again to 10.3 L m^{-2} h^{-1} and gradually decreased to 0.4 L m^{-2} h^{-1} at the maximal VCF of 28.5. The initial flux at lab scale (10.3 L m^{-2} h^{-1}) was significantly higher compared to the start flux at pilot scale (6.8 L.m^{-2}.h^{-1}) and this can be explained by the use of a new NFX membrane for the lab test and the difference in module design (spiral wound module vs. flat sheet membrane) and feed flow.

The evolution of concentrations of the ten selected phenolic compounds and quinic acid are shown as function of increasing VCF in Fig. 4. The phenolic compounds were progressively concentrated by the NFX membrane in both pilot and further lab scale testing. The concencentration of the ten selected phenolic compounds and quinic acid increased from 59.5 mg L^{-1} in the ethanol:water extract (feed) to 1256.1 mg L^{-1} in the final retentate, hence the polyphenols were concentrated by a factor 21.1. This increase in concentration is somewhat lower than the total VCF reached (28.5), pointing to some losses across the membrane to the permeate side (e.g. oxidation) and fouling on the membrane surface, but losses during sampling and draining of the test units may have occurred as well. This observation was not in accordance with Cissé *et al.* (2011), who showed a VCF of about 6 and the concentration of roselle extract from 4 to 25 g total soluble solids per 100 g, multiplying by 6 the anthocyanin concentration.

The average retention of the phenolic compounds was 97-98%, except for quinic acid (92%), catechin (78%) and epicatechin (87%). These observations are similar to the lab concentration tests and are expected given the MWCO of the NFX membrane of 150-300 Da.

In conclusion, phenolic compounds were efficiently concentrated from a hydro-alcoholic crude extract at pilot scale using a commercial membrane module, indicating the technical feasibility of nanofiltration for mild concentration of phenolic compounds.

Techno-economic assessment

The results of the pilot concentration test are directly integrated in the economic model to have good insight into the economic feasibility of the process. For the analyses we assumed a total input of ca. 5,000 ton apple pomace per year, resulting in ca. 3,800 kg of polyphenol. The total yearly energy use in the process amounted to approximately 3,400 MWh. This energy use resulted mainly from the extraction step. The total investment cost for the process amounts to 150,000 euro. The operational costs of 760,000 euro are higher than the yearly revenues of 105,000 euro. From this analysis it can be concluded that the resulting net present value (NPV) amounts to minus 5 million euro. This means the process is, under the assumptions made, not economically feasible. The total cost per kg polyphenol extracted is 203 euro under the assumptions made. Taking into account that the current market price for polyphenol is only 28 euro per kg, the costs for extracting polyphenols should be reduced drastically. Therefore, using an uncertainty analysis, we identified the most important parameters that determined the economic feasibility. If we only take into account the economic parameters, the ethanol use and electricity price are most important. These determine respectively 54% and 40% of the variance in the NPV. The price of phenolic compounds and the wage rate also influence the NPV, however, to a much smaller amount, i.e. 4% and 2% respectively. In a second analysis we took into account both technical and economic parameters. From this second analysis it is concluded that mainly the recycling rate of the ethanol has a large impact on the variation into the NPV. In future research these results should be taken into account. An optimization in the energy use has to be investigated. A decrease in the electricity price itself is only possible if the installation is exploited by a company with a high energy use. Possibly, another extraction solvent with a similar extraction yield and a lower price can be an option for improvement.

Conclusions

On lab scale, phytochemicals are often extracted with the use of organic solvents. In recent years, a trend towards the use of environmentally friendly extraction solvents and the use of food by-products as resource for extraction is emerging. This study showed at lab and pilot scale the extraction of phenolic compounds from apple pomace, an industrial by-product from the fruit juice industry, with ethanol:water (56%) as extraction solvent. However,

phytochemicals like polyphenols are present in relatively low concentration in the fruit matrix and concentration in the extraction solvent is necessary to develop an industrially relevant process. In this study, nanofiltration was selected for concentration due to its low energy consumption and mild processing conditions compared to other technologies. This study showed the technical feasibility of extraction and membrane based concentration of polyphenols from apple pomace at lab and pilot scale. Unfortunately, the extraction and concentration process was not economically feasible under the assumptions made. The electricity price and ethanol use had the highest influence on the economic feasibility of the full process under investigation.

These results suggest that research about polyphenol extraction from fruit by-products and membrane concentration processing should be continued to make the process economically feasible. An important point of attention for new research is the correlation of a detailed polyphenol analysis as performed in this study, with a total polyphenol analysis for example by the Folin-Ciocalteau colorimetric method, and with the antioxidant capacity since there is no consensus in literature on a positive correlation between total phenolics and anti-oxidant activity. In this way, the effect of nanofiltration on anti-oxidant activity of apple extract at lab and pilot scale can be studied.

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TABLE 1.

PROCESS PARAMETERS OF GRINDING SYSTEM (MULTICUT) AND SPIRAL-FILTER PRESS

Parameter	Unit	Value
Rotation speed screw Multicut	rpm	11.2
Rotation speed knives Multicut	rpm	1440
Number of teeth on knives	-	5
Inclination of spiral	degrees	38-25
Number of canals in spiral	-	4
Frequency of spiral	%	100
Frequency of feed pump	%	12
Frequency of vacuum pump	%	100
Pore size filter	µm	100
Absolute N_2 pressure in Multicut	bar	1.6
Absolute pressure in extraction cell	bar	0.15
Absolute pressure bottom of spiral	bar	1.1
Yield juice	% (w/w)	83.3
Throughput (mass flow apple)	kg h^{-1}	446

TABLE 2.

OVERVIEW OF USED MEMBRANES

Membrane	Manufacturer	Filtration application	MWCO (Da)[a]

DuraMem200	Evonik (Germany)	organic solvent (e.g. acetone)	200
Desal-5 DK	GE Osmonics (USA)	water	150-350
NFX	Synder Filtration (USA)	water	150-300
NF90	Dow Filmtec (USA)	water	n.a.[b]
DuraMem300	Evonik (Germany)	organic solvent (e.g. acetone)	300
NF030306	SolSep (the Netherlands)	organic solvent (e.g. acetone)	n.a.
NanoPro AS3012	AMS Technologies (Israel)	organic solvent (e.g. acetone)	180

641 [a] MWCO: molecular weight cut-off

642 [b] n.a.: not available

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665 TABLE 3.

666 NOMENCLATURE AND MOLECULAR WEIGHT (MW) OF TEN SELECTED

667 PHENOLIC MARKER COMPOUNDS AND QUINIC ACID IN APPLE FRUITS

Phenolic class	Name	Common name	MW (g mol^{-1})
Cyclitols	Quinic acid	Quinic acid	192
Hydroxycinnamic acids	*Trans*-3-caffeoylquinic acid	Chlorogenic acid	354
Flavonols	Quercetin-3-*O*-glucoside	Isoquercitrin	464
	Quercetin-3-*O*-galactoside	Hyperin	464
	Quercetin-3-*O*-rutinoside	Rutin	611
	Quercetin-3-*O*-arabinoside	Avicularin	434
	Quercetin-3-*O*-rhamnoside	Quercitrin	448
Dihydrochalcones	Phloretin-2'-*O*-glucoside	Phlorizin	436
Flavanols	(+)-Catechin	Catechin	290
	(−)-Epicatechin	Epicatechin	290
Procyanidins	(−)-Epicatechin-(4β → 8)-(−)-epicatechin	Procyanidin B2	579

TABLE 4.

690 AVERAGE CONCENTRATION OF PHENOLIC COMPOUNDS IN APPLE AND
691 AVERAGE FRACTIONATION IN JUICE AND POMACE AFTER AEROBIC AND
692 ANAEROBIC PRESSING (± STANDARD DEVIATION FOR N = 2)

	Concentration	Fractionation (%)			
	$\mu g\ g^{-1}$ DW	Aerobic pressing		Anaerobic pressing	
Phenolic compound	Apple	Juice	Pomace	Juice	Pomace
Quinic acid	1285 ± 7	48.6 ± 1.6	17.7 ± 1.7	65.2 ± 0.7	32.6 ± 1.7
Chlorogenic acid	1390 ± 0	38.5 ± 2.2	2.8 ± 0.1	61.2 ± 0.6	22.5 ± 4.5
Isoquercitrin	39 ± 1	8.3 ± 0.6	27.3 ± 2.0	11.9 ± 0.7	39.8 ± 1.4
Hyperin	217 ± 15	4.3 ± 0.3	29.5 ± 2.2	5.2 ± 0.4	42.6 ± 7.7
Rutin	10 ± 0	5.0 ± 0.2	22.4 ± 1.2	7.1 ± 0.2	32.6 ± 3.0
Avicularin	213 ± 14	6.8 ± 0.5	38.3 ± 3.0	10.0 ± 0.7	59.0 ± 11.3
Quercitrin	169 ± 8	9.2 ± 0.5	41.5 ± 2.0	18.7 ± 0.9	64.8 ± 11.2
Phlorizin	261 ± 69	27.5 ± 7.3	21.8 ± 5.8	51.3 ± 13.6	76.1 ± 21.2
Catechin	25 ± 1	47.1 ± 1.4	4.2 ± 0.2	84.8 ± 2.4	29.8 ± 1.2
Epicatechin	471 ± 13	43.0 ± 3.3	7.3 ± 0.2	75.0 ± 2.1	35.3 ± 4.3
Procyanidin B2	1020 ± 14	37.4 ± 1.8	4.2 ± 0.1	57.9 ± 0.8	20.4 ± 3.6
Total without quinic acid	3813 ± 75	32.7 ± 1.2	10.6 ± 0.2	52.9 ± 1.1	32.5 ± 2.3
Total with quinic acid	5098 ± 75	36.7 ± 1.0	12.4 ± 0.5	56.0 ± 0.9	32.5 ± 1.8

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706 TABLE 5.

AVERAGE CONCENTRATION OF PHENOLIC COMPOUNDS IN EXTRACTS FOR SCREENING TESTS (± STANDARD DEVIATION FOR N = 5)

Concentration (µg g^{-1} DW)	Screening tests	
Phenolic compound	Ethanol:water	Acetone:water
Quinic acid	498 ± 17	542 ± 51
Cathechin	18 ± 0	19 ± 1
Epicathechin	431 ± 34	474 ± 38
Chlorogenic acid	363 ± 12	421 ± 19
Avicularin	400 ± 21	448 ± 41
Phlorizin	566 ± 34	605 ± 96
Quercitrin	360 ± 16	414 ± 33
Hyperin	250 ± 9	291 ± 22
Isoquercitrin	46 ± 3	51 ± 3
Procyanidin B2	561 ± 30	687 ± 72
Rutin	11 ± 0	13 ± 1
Total without quinic acid	3007 ± 64	3423 ± 133
Total with quinic acid	3505 ± 66	3965 ± 142

TABLE 6.

MEMBRANE SCREENING TEST: TIME-AVERAGE PERMEATE FLUX OF SCREENED MEMBRANES AT STEADY-STATE CONDITIONS FOR THE ETHANOL:WATER AND ACETONE:WATER EXTRACTS

		Permeate flux (L m^{-2} h^{-1})	
Membrane	Pressure (bar)	Ethanol:water	Acetone:water
DuraMem200	20	11.9	17.0
DuraMem200	30	15.2	19.5
Desal-5 DK	20	13.6	n.d.
NFX	20	13.5	n.d.
NF90	20	22.7	n.d.
DuraMem300	20	n.d.[a]	16.0
NF030306	20	n.d.	1.1
NanoPro AS3012	20	n.d.	1.3

[a] n.d.: not determined

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