Role of Pectin and Haze Particles in Membrane Fouling during Cold Microfiltration of Apple Cider

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Abstract

The role of pectin in membrane fouling during cold MF of apple cider was investigated. Clarified apple juice with four different concentrations of pectin was subjected to cold MF, at pore sizes above 0.45 μm . The experimental data shows that pectin plays a significant role in membrane fouling, and its fouling effect increased with increasing concentration. The association of pectin with polyphenols and proteins, which results in colloidal haze particles with low surface electrical charge, seems to be the major factor in fouling during cold MF of apple cider. Depectinization was beneficial to MF with pore sizes below 0.45 μm , for which fouling is dominated by cake layer formation. Depectinization had a negative effect on MF flux for pore sizes above 0.8 μm , since the size reduction of haze particles accentuated pore constriction and blockage, which dominate fouling in large pore MF. These findings have practical implications for the development of efficient, commercially viable, cold MF processes for minimally processed apple cider.

Keywords: Cold Microfiltration; apple cider; membrane fouling; pectin; haze; depectinization

1 Introduction

Microfiltration (MF) is a pressure-driven membrane process that can be used to clarify liquid streams by removing suspended solids larger than the membrane pore size. MF is used in the manufacturing of apple juice primarily as an alternative to the conventional clarification methods such as filtration using sheets and diatomaceous earth. In addition to clarification, MF can remove many of the microorganisms of concern from the unfiltered apple juice (also known as “apple cider” in the US), including bacteria, yeast, mold and protozoa (Zhao et al., 2015a).

A significant issue that limits the commercial use of membrane filtration is membrane fouling. In MF, membrane fouling mechanisms include pore constriction, pore blocking and cake layer formation (de Barros, Andrade, Mendes, & Peres, 2003). Feed particles smaller than the membrane pore openings can get adsorbed onto the internal membrane channels and cause pore constriction, thus reducing the effective diameter of the pores. When particle diameters approach the effective membrane pore size, pore blocking can occur due to a convective drag of particles toward the membrane created by the permeate flux, and particles are adsorbed and/or deposited onto the membrane pores and surface. When particles are larger than membrane pores, they can be retained in a cake layer onto the membrane surface. A study that investigated the effect of pore size in membrane fouling during MF of apple cider revealed that the predominance of one of these mechanisms depends on the membrane pore size (Zhao and Moraru, 2015b).

In membrane filtration of apple cider, haze particles and pectin are believed to have a significant role in membrane fouling. While pectin is considered to be the individual haze component that contributes the most to membrane fouling, the structure and interactions of the haze components are most critical to fouling (Riedl, Girard, & Lencki, 1998; Su, Liu, & Wiley, 1993). Colloidal haze particles in apple cider are formed of proteins, polyphenols and pectin

(Beveridge & Wrolstad, 1997; Siebert, 2009). Proline-rich haze active proteins bind to polyphenols by hydrogen bonds and hydrophobic interactions (Siebert, Troukhanova, & Lynn, 1996). Pectin can also interact with polyphenols via hydrogen bonds, hydrophobic and electrostatic interactions (Le Bourvellec, Guyot, & Renard, 2009).

Much of the data available on the MF of apple cider focuses on MF with a pore size of 0.2 μm , also known as sterilizing MF. Membranes with such small pore sizes retain some of the components that contribute to juice color and flavor, such as pectin and chemical compounds, thus stripping the final product of some of its most desirable properties (Wu, Zall, & Tzeng, 1990). Therefore, using larger membrane pore sizes for clarification and microbial removal could result in increased retention of the nutritional, color and flavor components of the juice, as well as a potentially higher permeate flux as compared to 0.2 μm membranes. Nonetheless, the fouling mechanisms during large pore, cold MF of apple cider have not been yet fully elucidated. This study focuses specifically on understanding the role of haze particles and pectin in membrane fouling during large pore cold MF of apple cider. This can provide an insights into ways to mitigate fouling and make large pore size MF of apple cider a commercially viable process.

2 Materials and Methods

2.1 Apple cider

Raw apple cider was obtained from Cornell Orchards (Ithaca, NY) and stored at 4°C for a maximum of two weeks prior to use. Due to seasonality, some apple cider was stored frozen and thawed before being processed.

2.2 Pectin addition experiments

To evaluate the role of pectin in membrane fouling, a “base juice” was obtained by clarifying raw apple cider using a 0.2 µm pore size MF membrane, which helped remove most of the suspended solids. Apple pectin with molecular weight (MW) of 30,000-100,000 (Sigma Aldrich, St. Louis, MO) was added to this clarified juice, in a range of concentration typically found in apple cider. The target concentrations used were 0.05%, 0.10%, 0.15% and 0.20% w/w, denoted as concentration levels I to IV.

2.3 Pectinase treatments

The effect of pectin hydrolysis on fouling during apple cider MF was evaluated by conducting pectinase treatments on apple cider prior to MF. Three pectinases were pre-screened by monitoring their effect on the particle size in apple cider: commercial pectinase blend ClariSEB RL (Specialty Enzymes & Biotechnologies, CA), pectolyase (E.C. 3.2.1.15) (PL) from *Aspergillus japonicus* and polygalacturonase (E.C. 3.2.1.15) (PG) from *Aspergillus niger* (Sigma Aldrich, St. Louis, MO). PG was selected for further use. PG at a level of 0.012% (w/w) was added to raw cider, and the PG treated cider was kept for 6 days at 4°C prior to MF processing.

2.4 Microfiltration processing

A pilot scale MF unit consisting of a 50 gal (189.3 L) feed tank connected to a variable-speed centrifugal pump, a tubular heat exchanger and ISOFLUX™ tubular ceramic membrane of TAMI design (GEA Filtration, WI) placed inside a stainless steel housing was used. The membrane had an outside diameter of 25 mm, length of 1,200 mm, 23 internal channel of 3.5 mm hydraulic diameter each, and a membrane surface area of 0.35 m². The membrane pore sizes (the nominal pore diameter per the membrane manufacturer) used in this study were 0.2 µm (used only for clarification prior to pectin addition), 0.45 µm, 0.8 µm and 1.4 µm.

Feed inlet pressure (P_1), retentate outlet pressure (P_2) were recorded, and transmembrane pressure (TMP) was calculated as:

$$TMP = P_1 + P_2 - P_p \quad (1)$$

where permeate pressure (P_p) is the atmospheric pressure.

All pressures were expressed in kPa.

The permeate flux data was obtained gravimetrically using an electronic scale. Permeate flux (J) was calculated as:

$$J = \frac{M}{A \times t} \times \rho \quad (2)$$

where: J : permeate flux (L/m^2h); M : amount of permeate (kg) collected in the time interval t (hours); A : surface area of the membrane (m^2); ρ : density of the permeate at the filtration temperature (kg/m^3). The initial flux value was taken at 2 min after starting the pump, and every 5 s thereafter. To eliminate the “noise” generated by the multitude of data points, one point every 100 s was used when plotting the flux data.

The relative flux (J/J_0) was calculated as follows:

$$Relative \ flux = \frac{J}{J_0} \times 100\% \quad (3)$$

where: J : permeate flux at any time point (L/m^2h); J_0 : initial flux (L/m^2h).

This normalized parameter allows direct comparisons among MF experiments that have different permeate flux values. A lower J/J_0 indicates a more pronounced fouling of the membrane than a higher J/J_0 value.

The MF process was conducted at a cross-flow velocity of 5.5 m/s and a TMP of 159 kPa, which were selected based on optimized conditions from a previous study (Zhao and Moraru, 2015b). MF was conducted at a temperature of 6 ± 1 °C, which was maintained by circulating

chilled water in the counter current tubular heat exchanger. This temperature was chosen to preserve the quality of the juice and minimize browning during processing. Running MF cold also keeps the potential of a cold pasteurization process as an alternative to the conventional heat pasteurization (Zhao et al., 2015a).

2.5 Membrane cleaning

After each MF run, a chemical cleaning cycle was carried out. The cleaning procedure consisted of a rinse with reverse osmosis (RO) water for 10 min, followed by alkaline cleaning with Ultrasil-25 at a concentration of 20 g/L at 80 °C for 30 min and a second RO water rinse for 10 min or until neutrality. Acid cleaning with 5 mL/L HNO₃ at 50 °C for 20 min was then performed, followed by a third RO water rinse for 10 min or until neutrality. The effectiveness of cleaning and change in the membrane performance with time were monitored by determining the water flux of the clean membrane.

2.6 Physico-chemical analysis

The physico-chemical properties of the product were measured before and after MF. *pH* was measured at 20 °C using a Fisher Scientific Accumet Excel XL20 pH meter (Fisher Scientific, Pittsburgh, PA).

Soluble solids content (°Brix) was measured with a MISCO® digital probe refractometer (MISCO® Products Division, Cleveland, OH), at room temperature.

The *suspended insoluble solids (SIS)* content was determined by centrifuging 10 mL of apple cider or juice at 2200 x g for 15 min. After discarding the supernatant, the pellet, representing the SIS, was weighed and SIS calculated in g/L (Vaillant et al., 2008). All measurements were carried out in duplicate.

Viscosity was measured at 6°C using a Brookfield DV-II+ Pro viscometer with a ULV adapter (Brookfield Engineering Laboratories Inc., Middleboro, MA). Measurements were performed in triplicate.

Turbidity was measured using a 2020wi turbidimeter (LaMotte Company, Chestertown, MD) in Formazin Nephelometric Units (FNU). Measurements were performed in triplicate.

Pectin content was determined with a colorimetric assay using m-hydroxydiphenyl for the analysis of galacturonic acid (Kintner & van Buren, 1982). Each measurement was duplicated.

The *particle size distribution* was determined by dynamic light scattering using a Brookhaven 90Plus Particle Size Analyzer equipped with a Peltier temperature control system (Brookhaven Instruments Corporation, Holtsville, NY) at 20 °C, a fixed angle of 90°, and a wavelength of 658 nm. Data collection and analysis were performed using the BIC software (Brookhaven Instruments Corp., Holtsville, NY). The dust filter cut-off was set at 30, which improves the quality of the measurements by rejecting measurement resulted from random particles, such as air bubbles or dust. This value was selected based on the manufacturer recommendation for scenarios where the expected average particle size is in the range of hundreds of nm. The relative particle size distribution and the intensity weighted effective diameter were determined for each sample. It is important to note that this particle size measurement method uses the assumption that all particles have spherical shapes. No dilutions were made to the samples prior to particle size analyses. Each particle size measurement consisted of 8 individual runs for duration of 30 s per run. At least one measurement for each sample was conducted, and all measurements were taken within 24 h from processing.

The *zeta potential* of apple cider and juice was measured using the Zeta Potential attachment of the Brookhaven 90Plus Particle Size Analyzer; measurements were taken within 24 h from processing, in triplicate.

2.7 Statistical analysis

Data was analyzed using the statistical software JMP Pro 10 (SAS Institute, 2010). Student's t test at a significant level of 5% was used to determine statistical significance of observed differences among means.

3 Results and Discussion

3.1 Effect of pectin concentration on MF and fouling

3.1.1. Effect of pectin addition on juice properties

In a previous study it has been shown that fouling mechanisms in MF of apple cider are transitioning from the predominance of surface fouling for 0.2 μm and 0.45 μm pore sizes to the dominance of internal fouling for 0.8 μm and 1.4 μm (Zhao and Moraru, 2015b). Based on these conclusions, the effect of pectin concentration on permeate flux and fouling was investigated for one pore size from each group: 0.45 μm as a representative for “low pore size MF” and 0.8 μm for “large pore size MF”. These pore sizes were also chosen based on their potential to significantly reduce microbial pathogenic and spoilage organisms in apple cider (Zhao et al., 2015a).

Commercially available apple pectin was added to a “base juice”, which was obtained by clarifying apple cider using a 0.2 μm pore size membrane. This ensured that most of the soluble components of apple cider are maintained in the “base juice”. Four different levels of pectin addition were used (I to IV), which covered a range of pectin concentration typically found in apple cider. The actual pectin concentrations are shown in Table 1. The addition of pectin to the

base juice did not induce any changes in the pH or °Brix of the juice (Table 1), as expected. It is important to note that most of the added pectin passed through the MF membranes. As seen in Fig. 1, the 0.8 µm pore size allowed for a higher level of transmission than the 0.45 µm membrane. A linear regression analysis indicates a less than 10% reduction of pectin in the former and slightly over 20% reduction in the latter. Most of the pectin reduction occurred at the highest level of pectin addition (level IV), and the reasons for this will be discussed next.

An evaluation of the particle size of a pectin dispersion in distilled water indicated the presence of two distinct populations of particles (Fig. 2, upper): a class of particles with an average diameter smaller than 500 nm, and a class of micrometer sized particles, presumably pectin aggregates. It should be noted that since the particle size data in Figure 2 is based on the signal intensity, the distribution cannot be related quantitatively to the amount of pectin in each size class. This is consistent with previous studies, which reported two groups of particle sizes in apple cider, with a center of distribution of about 1 µm and about 5 µm, respectively (Genovese & Lozano, 2000; Beveridge, 2002; Zhao and Moraru, 2015b). Two classes of particles were also found in the juice with added pectin. While the small particles were in the same size range, regardless of the level of pectin addition, the larger particles increased in size as the pectin addition level increased. This is indicated by both a shift of the large particles towards a larger size, and an increase of the contribution of the large particles to the signal intensity for the dynamic light scattering analysis, as suggested by a more pronounced second peak (large particles) and a decrease in intensity of the first peak (small particles). This suggests an aggregation of pectin molecules. The propensity of pectin to form inter-molecular aggregates by hydrophobic and hydrogen bonds, particularly at low temperature, low pH and high concentration, has been reported before (Lopes da Silva and Rao, 2006). An increase in

aggregation with increasing pectin concentration can also be observed in Fig. 3, which shows an increase in the average particle diameter in the juice with added pectin (prior to MF) with concentration of added pectin. The trendlines in Fig. 3 were included for visual guidance only.

MF resulted in a significant decrease in particle size, due to the removal of large particles. Regardless of the added pectin level, juices filtered with a 0.45 μm pore size membrane had a particle size very close to the pore size of the membrane, and the highest particle size measured was 0.576 μm . For MF using a 0.8 μm pore size, the particle size of the MF juice followed a trend very similar to the juice prior to MF: particle size increased linearly with pectin concentration up to about 100 mg/ 100 g, and then reached a plateau, at a particle size of about 1 μm . The limit particle size found for both MF pore sizes was close, but slightly higher than the nominal pore size of the membranes. This could be due to some intermolecular aggregation that occurred in the MF juice, immediately after MF. This could involve either pectin-pectin interactions or pectin-polyphenol interactions.

The pectin concentration and particle size had a direct effect on some physicochemical properties of the MF juice, particularly turbidity and viscosity. First, the relationship between particle size and pectin concentration was directly mirrored by the relationship between turbidity and pectin concentration (Fig. 4). This was to be expected because particles in the micrometer range are directly responsible for scattering light and thus the higher the proportion of such particles in juice, the higher its turbidity.

Pectin concentration also impacted directly the shear viscosity of the juice, with viscosity increasing linearly with pectin concentration (Fig. 5). This type of relationship was observed both for the base juice with added pectin and for the MF juice, although the correlation became less strong after MF. This was mostly due to a weaker correlation in the high pectin

concentration range, caused by the removal of some of the large particles during the MF process. The only statistically significant change in viscosity after MF occurred for the highest pectin addition (level IV) ($P < 0.05$). At the highest level of pectin addition, an average drop in juice viscosity of 23% was observed for MF with 0.45 μm pore size, and a drop of 7% for MF with 0.8 μm pore size. These values are consistent with those obtained in the previous study performed on apple cider with a natural pectin concentration, where a drop in viscosity of 23.95% was reported for 0.45 μm pore size and 7.51% for 0.8 μm pore size (Zhao and Moraru, 2015b).

The repulsive electrostatic and hydration interaction forces between the dispersed colloidal particles in apple juice contribute significantly to the viscosity of cloudy apple juice (Genovese & Lozano, 2006), and their removal by MF can result in a decrease in viscosity. The decrease in charge of juice after MF was confirmed by zeta potential (ζ) analyses. As seen in Fig. 6, the ζ measured in juice with added pectin prior to MF was in the range -10 to -15 mV, similar to the values reported by Benitez and Lozano (2006) for apple juice of about 12 °Brix. Pectin was the major contributor to the negative charge of the juice, as ζ of the base juice was negligible for most samples (Fig. 6). A clear decrease in the magnitude of ζ was observed after MF, particularly for the 0.45 μm pore size (Fig. 6, bottom). By correlating the particle size, viscosity and zeta potential data, it can be concluded that this was caused by the removal of negatively charged, large pectin aggregates.

3.1.2. Effect of pectin addition on MF flux and membrane fouling

Pectin addition to the base juice had a profound effect on the MF permeate flux. Flux decreased with increasing pectin concentration in the feed for both pore sizes (Fig. 7). Similar results were reported in a previous study that used a model fluid comprised of pectin, sugar and citric acid (Su, Liu & Wiley, 1993). This could be attributed to the effect of feed viscosity on

permeate flux, since flux is inversely related to feed viscosity. Fig. 7 shows that the relationship between feed viscosity and permeate flux follows a power law model, both for the initial flux and for flux at 20 min. It should be noted that permeate flux for 0.8 μm pore size was higher than for 0.45 μm pore size, at all pectin concentrations. This demonstrates a clear influence of pectin on membrane fouling during MF of apple juice / cider.

Nonetheless, pectin is not the only juice component responsible for fouling. When comparing the MF flux curves for juice with added pectin and apple cider with natural composition, at a similar pectin content (Fig. 8), two observations can be made. First, the permeate flux decreased with pectin content for both types of feed. Second, MF of apple cider had three to four fold lower fluxes than MF of base juice with added pectin, for both MF pore sizes. This indicates that besides the actual pectin content, the other juice components, particularly polyphenols and proteins, and their interactions with pectin, have an even larger contribution to fouling. In particular, the formation of haze particles by the interaction of negatively charged pectin with proteins, which are positively charged at the apple juice pH, leads to the formation of haze particles (Yamasaki et al., 1967). This also explains the very low charge, indicated by the small ζ values of the juice without added pectin (Fig. 6) and in the untreated apple cider (Table 2). The ζ of the ceramic membrane, measured in a previous study (Zhao et al., 2015b) is -8.53 ± 1.18 mV. It can be therefore inferred that the negatively charged particles in the juice with added pectin will be rejected stronger by the negatively charged membrane and will also repel each other electrostatically, thus contributing less to membrane fouling. On the other hand, the almost neutral haze particles in the untreated apple cider are capable of depositing onto the membrane surface and form a densely packed fouling layer, which explains the much lower permeate flux for cider as compared to the juice with added pectin, even at similar pectin levels.

3.2 Effect of depectinization on MF and fouling

Previous studies have shown that depectinization can improve the performance of membrane filtration of apple cider using small pore sizes (Su, Liu, & Wiley, 1993; Wu, Zall, & Tzeng, 1990; Padilla-Zakour & McLellan, 1993), but the effect of depectinization for pore sizes above 0.2 μm has not yet been reported. In this study, the effect of depectinization on MF of apple cider with large pore sizes (0.45, 0.8 and 1.4 μm) was investigated. Three different enzymes were tested for effectiveness of depectinizing apple cider, and the physicochemical properties of cider before and after treatment with these enzymes are shown in Table 2. Polygalacturonase (PG) showed the largest reduction in particle size and was chosen for subsequent treatments.

During depectinization the enzyme attacks the pectin coat of the haze particles, exposing the positively charged protein core and allowing electrostatic aggregation of haze particles (Yamasaki et al., 1967; Beveridge, 1997). Although no changes in ζ were observed after the enzymatic treatment performed here (Table 3), this measurement only captured the net result of the depectinization treatment. A reduction in particle size as a result of the PG treatment was observed and, although this was not drastic, it indicates that the aggregates present in the depectinized cider are likely different than in the untreated cider, due to the enzymatic treatment. The pectin content measured in the apple cider after pectinase treatment was less than 50% of the pectin content prior to the PG treatment (Table 3), which suggests that some of the pectic materials may have precipitated out of solution during the enzymatic treatment. This is also supported by the observed increase in SIS after the PG treatment. The other characteristics of apple juice have not significantly changed after the enzymatic treatment (Table 3).

MF of depectinized apple cider resulted in visually clear apple juice, and a complete removal of SIS (Table 3). Yet, some differences in the instrumentally measured turbidity were found: MF juice obtained from PG treated cider had a lower turbidity than the juice obtained from untreated cider, especially for the 0.8 μm and 1.4 μm pore sizes (Fig. 9). This correlates well with the fact that the MF juice from PG treated cider had significantly smaller particle sizes compared to the MF juice from untreated cider, for both 0.8 μm and 1.4 μm pore sizes (Fig. 10).

PG treatment also caused a significant decrease in viscosity, from an average of 3.18×10^{-3} Pa·s for the untreated apple cider to an average of 2.59×10^{-3} Pa·s for the PG treated cider. No difference was found between the viscosity of the PG treated cider and of the MF juice obtained from this cider. The decrease in viscosity after PG treatment was expected to have a positive effect on the subsequent MF process, since a lower viscosity promotes mass transfer through the membrane. PG treatment of the cider caused a significant increase in flux for the 0.45 μm pore size membrane (Fig. 11), which is in accordance with previous studies (Su, Liu, & Wiley, 1993; Wu, Zall, & Tzeng, 1990; Padilla-Zakour & McLellan, 1993). However, the opposite was observed for MF of PG treated cider using the 0.8 μm and 1.4 μm membranes, for which the flux was significantly lower than for the MF of untreated cider (Fig. 11). Fig. 12 shows that fouling rate, expressed by the relative drop in flux, was more pronounced after PG treatment for both 0.45 μm and 1.4 μm pore sizes.

The results could be explained by the different fouling mechanism in small (0.45 μm) and large membranes (0.8 and 1.4 μm). It was proposed before that fouling in MF of apple cider using 0.45 μm membranes is dominated by cake layer formation, while for 0.8 and 1.4 μm is dominated by pore constriction and pore blocking (Zhao and Moraru, 2015b). Most likely, the reduction of haze particle size by PG treatment resulted in increased pore constriction and

blocking for the 0.8 μm and 1.4 μm membranes, and further reduced the effective pore size of these two membranes. However, for the 0.45 μm pore size membrane, the breakdown of pectin molecules by PG weakened the association within the haze particles, which may have led to a less dense fouling layer, with a lower resistance to flow. This is supported by the conclusions of Yu & Lencki (2004), who showed that depectinized cider led to larger, more open pore structure deposits on membranes as compared to untreated cider.

Contrary to what was anticipated, depectinization does not appear to be beneficial for large pore MF (pore sizes above 0.8 μm), but it leads to improved fluxes in small pore size MF (pore size below 0.45 μm).

4 Conclusions

Pectin does play a significant role in fouling during cold MF of apple cider, and its fouling effect increases with higher concentrations. Most significant is pectin's association with other components in apple cider, particularly proteins and polyphenols, which leads to haze particles with low surface electrical charge. Such particles seem to be the main contributor to membrane fouling during cold MF of apple cider. Pectinase treatment resulted in size reduction and structural changes of haze particles, which were beneficial to MF with membrane pore sizes below 0.45 μm . However, depectinization did not have a positive effect on MF using pore sizes above 0.8 μm , since the size reduction of haze particles accentuated fouling by pore constriction and blockage, which are the specific fouling mechanisms in large pore MF of apple cider. It is therefore suggested that depectinization of apple cider prior to cold MF should only be used for small pore size MF (<0.45 μm), where this treatment can help reduce fouling and achieve a commercially viable process performance for minimally processed apple cider.

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TABLES

Table 1. Physico-chemical properties of juice with the addition of different concentrations of pectin, before and after microfiltration (MF). Values represent averages of two runs (with 3 analytical replicates per run) $\pm$ 1 stdev

MF pore size	Pectin addition level	Pectin content before MF (mg/100 g)	pH		Soluble solids (°Brix)	
			Before MF	After MF	Before MF	After MF
0.45 μ m	0 (base juice)	4.16 $\pm$ 1.13	3.81 $\pm$ 0.04	3.84 $\pm$ 0.04	11.29 $\pm$ 0.06	11.46 $\pm$ 0.06
	I	36.49 $\pm$ 4.82	3.77 $\pm$ 0.11	3.78 $\pm$ 0.11	11.58 $\pm$ 0.12	11.54 $\pm$ 0.06
	II	71.70 $\pm$ 1.97	3.84 $\pm$ 0.07	3.86 $\pm$ 0.10	11.63 $\pm$ 0.06	11.75 $\pm$ 0.24
	III	130.99 $\pm$ 34.79	3.81 $\pm$ 0.02	3.85 $\pm$ 0.01	11.67 $\pm$ 0.24	11.58 $\pm$ 0.24
	IV	136.34 $\pm$ 1.58	3.75 $\pm$ 0.01	3.74 $\pm$ 0.03	11.71 $\pm$ 0.06	11.92 $\pm$ 0.12
0.8 μ m	0 (base juice)	16.35 $\pm$ 7.70	3.64 $\pm$ 0.16	3.69 $\pm$ 0.13	12.50 $\pm$ 1.41	12.50 $\pm$ 1.30
	I	56.12 $\pm$ 3.87	3.72 $\pm$ 0.07	3.74 $\pm$ 0.10	13.63 $\pm$ 0.29	13.79 $\pm$ 0.06
	II	86.70 $\pm$ 22.54	3.64 $\pm$ 0.07	3.67 $\pm$ 0.08	12.33 $\pm$ 1.18	12.46 $\pm$ 1.12
	III	102.59 $\pm$ 20.56	3.63 $\pm$ 0.04	3.63 $\pm$ 0.03	12.08 $\pm$ 0.12	12.13 $\pm$ 0.18
	IV	175.05 $\pm$ 31.44	3.67 $\pm$ 0.02	3.68 $\pm$ 0.02	12.21 $\pm$ 0.41	12.33 $\pm$ 0.82

Table 2. Physico-chemical properties of apple cider, before and after depectinization with various enzymes. Values represent averages of three analytical replicates $\pm$ 1 standard deviation

Sample	pH	Brix	Suspended insoluble solids (SIS) (g/l)	Zeta potential (mV)	Viscosity ($\times 10^{-3}$ Pa·s)	Turbidity (FNU)	Particle size (nm)
Untreated cider	3.96	12.0	20.88 $\pm$ 0.71	-0.65 $\pm$ 0.23	2.62 $\pm$ 0.01	1185 $\pm$ 5	1097 $\pm$ 29
Cider treated with Lyase	3.92	12.0	21.22 $\pm$ 0.16	-0.70 $\pm$ 0.12	2.50 $\pm$ 0.10	1124 $\pm$ 16	929 $\pm$ 72
Cider treated with commercial enzyme	3.92	12.5	17.63 $\pm$ 1.12	-0.75 $\pm$ 0.38	2.70 $\pm$ 0.04	1125 $\pm$ 9	1051 $\pm$ 115
Cider treated with Sigma PG	3.93	12.0	20.79 $\pm$ 1.81	-0.65 $\pm$ 0.27	2.69 $\pm$ 0.03	1146 $\pm$ 12	865 $\pm$ 106

Table 3. Physico-chemical properties of apple cider (before MF) and apple juice (after MF) in the depectinization study. Values represent averages of three runs (with 3 analytical replicates per run) $\pm$ 1 sdte

Property	Treatment	0.45 μ m MF pore size		0.8 μ m MF pore size		1.4 μ m MF pore size	
		Untreated	PG treated	Untreated	PG treated	Untreated	PG treated
Pectin content (mg/100 g)	Before MF	109.96 $\pm$ 109.30	44.89 $\pm$ 27.65	83.67 $\pm$ 31.73	38.21 $\pm$ 14.21	89.75 $\pm$ 36.37	40.30 $\pm$ 0.70
	After MF	21.91 $\pm$ 31.96 (21% transmission)	35.66 $\pm$ 21.27 (80% transmission)	75.18 $\pm$ 34.79 (86% transmission)	28.49 $\pm$ 5.76 (77% transmission)	70.96 $\pm$ 36.56 (84% transmission)	38.22 $\pm$ 5.90 (95% transmission)
pH	Before MF	3.69 $\pm$ 0.21	3.73 $\pm$ 0.01	3.74 $\pm$ 0.01	3.78 $\pm$ 0.00	3.70 $\pm$ 0.14	3.76 $\pm$ 0.01
	After MF	3.72 $\pm$ 0.18	3.74 $\pm$ 0.01	3.73 $\pm$ 0.19	3.81 $\pm$ 0.06	3.71 $\pm$ 0.14	3.77 $\pm$ 0.03
Soluble solids (°Brix)	Before MF	12.96 $\pm$ 0.69	14.00 $\pm$ 0.00	12.78 $\pm$ 0.15	13.83 $\pm$ 0.24	12.64 $\pm$ 0.51	13.75 $\pm$ 0.30
	After MF	13.20 $\pm$ 0.78	14.25 $\pm$ 0.35	13.11 $\pm$ 0.22	13.63 $\pm$ 1.24	13.03 $\pm$ 0.51	14.25 $\pm$ 0.30
Suspended insoluble solids (SIS) (g/L)	Before MF	18.50 $\pm$ 3.44	22.37 $\pm$ 3.65	19.34 $\pm$ 2.03	27.04 $\pm$ 2.91	19.04 $\pm$ 4.29	25.30 $\pm$ 2.09
	After MF	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Viscosity at 6°C ($\times 10^{-3}$ Pa·s)	Before MF	3.09 $\pm$ 0.72	2.65 $\pm$ 0.08	3.33 $\pm$ 0.60	2.54 $\pm$ 0.16	3.09 $\pm$ 0.69	2.58 $\pm$ 0.14
	After MF	2.35 $\pm$ 0.28	2.47 $\pm$ 0.08	3.08 $\pm$ 0.61	2.44 $\pm$ 0.19	2.96 $\pm$ 0.52	2.44 $\pm$ 0.12
Turbidity (FNU)	Before MF	922 $\pm$ 292	1661 $\pm$ 45	920 $\pm$ 257	2024 $\pm$ 612	927 $\pm$ 297	1617 $\pm$ 74
	After MF	1.65 $\pm$ 0.64	0.58 $\pm$ 0.01	55.10 $\pm$ 18.68	4.68 $\pm$ 1.06	54.03 $\pm$ 11.43	11.12 $\pm$ 0.30
Particle size (nm)	Before MF	1120 $\pm$ 479	958 $\pm$ 3	1027 $\pm$ 614	1318 $\pm$ 160	1035 $\pm$ 334	1137 $\pm$ 99

After MF	345±132	0.58±0.01	151±143	173±235	641±1	374±1
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FIGURES

Figure 1: Pectin transmission through the membrane after microfiltration (MF) with 0.45μm and 0.8 μm pore sizes. Data points correspond to two processing runs per pore size. Individual data points represent averages of three analytical replicates ± 1 stdev

Figure 2: Examples of particle size distribution for a dispersion of pectin in distilled water (upper) and for base juice with added pectin at levels I through IV (lower), prior to microfiltration (MF)

Figure 3: Relationship between particle size and pectin concentration in juice before and after microfiltration (MF). Data points correspond to two processing runs per pore size. Individual data points represent averages of three analytical replicates ± 1 stdev

Figure 4: Relationship between turbidity and pectin concentration in juice before and after microfiltration (MF).

Data points correspond to two processing runs per pore size. Individual data points represent averages of three analytical replicates ± 1 stdev

Figure 5: Shear viscosity (at 6°C) of juice before and after microfiltration (MF) as a function of pectin concentration. Data points correspond to two processing runs per pore size. Individual data points represent averages of three analytical replicates ± 1 stdev

Figure 6: Zeta potential measured in juice before and after microfiltration (MF) with 0.8 μm (top) and 0.45 μm (bottom), at different levels of pectin addition (I through IV). Data points correspond to two processing runs per pore size (1 and 2). Individual data points represent averages of three analytical replicates ± 1 stdev

Figure 7. Relationship between permeate flux and pectin concentration in the juice prior to microfiltration(MF).

Data points correspond to two processing runs per pectin addition level and pore size

Figure 8. Flux curves for MF of apple cider with natural composition and microfiltration (MF) of apple juice with added pectin, at similar pectin concentration, using (a) 0.8 μm membrane pore size and (b) 0.45 μm membrane pore size

Figure 9. Turbidity of microfiltered (MF) juice obtained from untreated and PG treated apple cider. Data points not connected by the same letter are statistically different from each other ($P < 0.05$)

Figure 10. Average particle diameters in untreated apple cider, microfiltered (MF) juice from untreated cider and MF juice from PG treated cider. Data points not connected by the same letter are statistically different from each other ($P < 0.05$)

Figure 11. Initial flux for microfiltered (MF) of untreated and PG treated apple cider. Data points not connected by the same letter are statistically different from each other ($P < 0.05$)

Figure 12. Relative flux after 1h for microfiltered (MF) of untreated and PG treated apple cider. Data points not connected by the same letter are statistically different from each other ($P < 0.05$)