DEVELOPMENT OF A NON-THERMAL HURDLE TECHNOLOGY FOR COLD PASTEURIZATION OF APPLE CIDER - A FOCUS ON MICROFILTRATION

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The effectiveness of a combined microfiltration (MF) and ultraviolet (UV) process on reduction microbial in apple cider was evaluated against pertinent pathogens and spoilage bacteria. MF using 0.8 µm and 1.4 µm pore size ceramic membranes was performed at 10 °C and a transmembrane pressure (TMP) of 155 kPa. The subsequent UV treatment was conducted at a low dose of 1.75 mJ/cm^2. The combined MF and UV process achieved more than 5 log reduction of *Escherichia coli*, *Cryptosporidium parvum* and *Alicyclobacillus acidoterrestris*, for both membrane pore sizes. MF with 0.8 µm pore size performed better than 1.4 µm pore size on the removal of *E. coli* and *A. acidoterrestris*. The developed non-thermal hurdle treatment has the potential to significantly reduce pathogenic and spoilage organisms in apple cider, and help juice processors improve the safety and quality of their products.

An optimization study concluded that a cross-flow velocity of 5.5 m/s and a TMP of 159 kPa are the optimal conditions for obtaining a high flux and low flux decline in cold MF of apple cider. A subsequent study was conducted to evaluate the effect of apple cider characteristics and membrane pore size on MF flux, under optimal MF conditions. Four membrane pore sizes were studied: 0.2, 0.45, 0.8 and 1.4 µm. MF using pore sizes of 0.2 µm and 0.45 µm on one hand, and 0.8 µm and 1.4 µm on the other hand, resulted in similar particle sizes and turbidity of the MF juice, and similar flux behaviors. In order to clarify the role of pectin in fouling during MF of

apple cider, pectin was added to a clarified base juice, at levels similar to those naturally occurring in apple cider. As pectin concentration increased, MF flux decreased, due to a large extent to the increase in viscosity with increasing pectin concentration. The MF flux for apple juice with added pectin was much higher than for untreated apple cider at the same pore size and a similar pectin concentration. This suggested that besides pectin concentration, the interaction of pectin with other juice components such as proteins and polyphenols and the formation of haze particles also have a significant contribution to fouling. Depectinization of apple cider improved MF flux using a 0.45 μm membrane pore size, but had an adverse effect on the flux for 0.8 μm and 1.4 μm pore sizes. This could be explained by the different fouling mechanisms for different membrane pore sizes. Fouling for MF with 0.2 μm and 0.45 μm pore sizes is dominated by surface fouling and cake layer formation, while fouling for MF with 0.8 μm and 1.4 μm is dominated by pore constriction and pore blocking. It was proposed that the fouling mechanism depends on the relative size of membrane pores and the particles suspended in the feed, and there is a critical pore size where fouling transitions from the dominance of surface fouling and cake layer formation to the dominance of pore constriction and pore blocking.

The conclusions of this work will help clarify some of the fundamental questions related to MF of apple cider, and at the same time offer some practical solutions for optimization and maximization of performance during MF of apple cider.

BIOGRAPHICAL SKETCH

Dongjun Zhao is originally from Shenyang, Liaoning, China. She received her Bachelor's Degree in 2006 and Master's Degree in 2008 from the Department of Food Science at Cornell University. Dongjun began her Ph.D. program with Dr. Carmen Moraru at Cornell University in August 2010 after working in the food industry for two years. She has been involved in numerous extracurricular and professional development activities, including leading and participating in several product development competitions, College Bowl team, Phi Tau Sigma Honor Society for Food Science and Technology, Western New York IFT, and the Graduate Seminar Committee. The teams she led or participated in won second and first places in the IFTSA Mars Product Development Competition in 2011 and 2012. She was also a co-inventor of a patented process technology developed during the 2011 competition and the technology was licensed in 2014. Dongjun also led a team in the national Dairy Research Institute New Product Competition and the entry was selected as one of six finalists. She dedicated numerous hours to mentoring three undergraduate research assistants and one M.P.S. student during her Ph.D. study. Two of the undergraduate students completed their honor theses and one of them was selected as the Finalist in the 2012 IFTSA Undergraduate Research Competition. While at Cornell, Dongjun received IFT Feeding Tomorrow Scholarship, Ruth Herzog and Albert Flegenheimer Memorial Graduate Fellowship, and Unilever United States Graduate Award.

This dissertation is dedicated to my beloved parents, Fengxian Wang and Lianyu Zhao, who give me unconditional love and without whom I would not be the person I am today; to my husband, Chris A. Johnson, for all your love, patience and support; to my beautiful baby Brendan Zhao Johnson, who is the light of my life.

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LIST OF ABBREVIATIONS

E. coli	*Escherichia coli*
C. parvum	*Cryptosporidium parvum*
A. acidoterrestris	*Alicyclobacillus acidoterrestris*
MF	Microfiltration
UV	Ultraviolet
NTU	Nephelometric Turbidity Unit
TSA	Trypticase Soy Agar
TSB	Trypticase Soy Broth
CFU	Colony Forming Units
PCA	Plate Count Agar
PCR	Polymerase Chain Reaction
RT-PCR	Real Time Polymerase Chain Reaction
HBSS	Hanks Balanced Salt Solution
Ct	Cycle Threshold
APDA	Acidified Potato Dextrose Agar
BPB	Butterfield's Phosphate Buffer
TMP	Transmembrane Pressure
RO	Reverse Osmosis
TA	Titratable Acidity
SIS	Suspended Insoluble Solids
ULV	Ultra Low Viscosity
FNU	Formazin Nephelometric Turbidity Unit
SEM	Scanning Electron Microscopy
PG	Polygalacturonase

PL	Pectolyase

LIST OF SYMBOLS

P1	Feed Inlet Pressure
P2	Retentate Outlet Pressure
Pp	Permeate Pressure
ζ	Zeta Potential
J	Permeate Flux (L/m^2h)
M	Amount of Permeate (L) Collected in the Time Interval *t* (hours)
t	Time (hours)
A	Surface Area of the Membrane (m^2)
ρ	Density of the Permeate at the Filtration Temperature (kg/m^3)
J_o	Initial Flux (L/m^2h)
ΔP_{tm}	Transmembrane Pressure
η	Viscosity ($Pa \cdot s$)
R_t	Total Hydraulic Resistance (m^{-1})

CHAPTER 1

JUSTIFICATION

Apple juice and cider are very important agricultural commodities in the U.S. In 2010, 31% of the U.S. apple crops were used for processed products, out of which 43.6% on a volume basis was represented by juice, which equaled a farm-gate value of $98 million (U.S. Apple Association, 2011). Apple juice was the second leading flavor (12.3% in volume) of the U.S. juice market in 2010 (Agriculture and Agri-Food Canada, 2012), following orange juice. In 2010, the U.S. apple juice and cider production was 1,336 million pounds (ERS, 2012).

In recent years, the consumers' demand for high quality, non-heat treated, minimally processed apple juice has increased significantly. This sometimes posed a safety risk, as consumers are sometimes drawn to raw juice or cider products. Consumption of raw apple juice or cider contaminated with *Escherichia coli* O157:H7 and *Cryptosporidium parvum* has resulted in several outbreaks and has raised public health concerns since the 1990s (Besser, 1993; Centers for Disease Control and Prevention (CDC), 1997; Cody and Glynn, 1999; Diallo et al., 2011; Hilborn et al., 2000; Millard, 1994; Vojdani et al., 2008). To address the safety concern, the U.S. Food and Drug Administration (FDA) mandates that all juice processors implement the HACCP system in their production and that a 5-log reduction in target pathogens needs to be achieved during manufacturing of apple juice and cider (U.S. Food and Drug Administration, 1999).

To make 100% apple juice, apple fruits go through crushing, pressing, collection of juice, pasteurization, enzymatic treatment, clarification, storage and packaging. FDA requires thermal pasteurization of apple juice and cider at 71.1°C for at least 6 seconds. Thermal treatment can achieve a 5-log reduction of pertinent pathogens, *Escherichia coli* O157:H7 and

Cryptosporidium parvum, but it can have some negative effects on the nutritional and organoleptic properties of juice. To minimize such changes, ultraviolet (UV) treatment has been developed as a non-thermal alternate processing method for apple juice and cider. Studies have shown and validated that UV irradiation can achieve 5-log reduction of *Escherichia coli* O157:H7 and *Cryptosporidium parvum* (Basaran et al., 2004; Hanes et al., 2002; Quintero-Ramos et al., 2004). In 2000, UV was approved by FDA as an alternative to thermal pasteurization of apple juice and cider. Nevertheless, high doses of UV can negatively affect juice color and flavor, while suspended solids in cider can limit UV treatment efficiency. One study showed that UV dose larger than 10.62 J/cm^2 using a rising film reactor had negative effects on juice quality in terms of color and flavor (Caminiti et al., 2012). In addition, yeasts and molds are not affected by UV treatment, thus result a shorter shelf life of juice and cider, usually 2 weeks, than thermal treated product (Azhuvalappil et al., 2010).

Microfiltration (MF) has been widely used in the juice industry for clarification. It operates on the principle of physically removing particles that are larger than its membrane pore sizes (sieving effect). To overcome the limitations of UV treatment, removing some of the suspended solids, including yeast and molds by MF before UV treatment could offer the benefits of extended shelf life and lower UV dose required to achieve 5-log reduction of pertinent pathogens, while maintaining the organoleptic properties of juice. Conducting MF at low temperatures will further ensure that the freshness of the processed juice is maintained. Combining low temperature MF and UV treatment in a non-thermal hurdle treatment can lead to a viable alternative to the processes currently used by the juice industry. Nonetheless, limited data exists in literature regarding the effectiveness of and pathogen removal efficiency of cold MF in general, and of MF using pore sizes larger than 0.2 μm in particular. A significant issue

that affects membrane filtration, including MF, and limits its large scale commercial application, is membrane fouling. The fouling mechanisms in apple cider MF are not well understood. A good and clear understanding of these mechanisms might provide an insight into ways to mitigate fouling efficiently. Although the juice industry uses sometimes empirical means of alleviating fouling, including pre-treatment with pectinase, heat shock, and gelatin addition before MF, these are not always very effective and some of them are also costly. Therefore, there is both a need and an opportunity for the development of new methods for mitigating fouling in apple cider MF. The elucidation of fouling mechanisms and the development of antifouling methods are both of scientific and practical interest, as they could help improve the MF process efficiency and make it economically feasible for juice processors.

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CHAPTER 2

RESEARCH OBJECTIVES

As a direct response to the research needs described, the goal of this research is to better understand the factors that affect the efficiency and fouling in cold MF of apple cider, and integrate MF in a hurdle, non-thermal treatment able to achieve a 5-log reduction of the pathogens of concern for apple juice and cider, while maintain the quality and freshness of the processed product.

The following specific objectives are proposed:

1. Development of a non-thermal MF and UV combination treatment for the efficient reduction of pathogens and spoilage bacteria from apple cider
2. Investigation of MF fouling mechanisms and process optimization
   2.1. Optimization of large pore microfiltration of apple cider
   2.2. The effect of apple cider characteristics and membrane pore size on membrane fouling
   2.3. Role of haze particles and pectin in membrane fouling formation
   2.4. Optimization of a CO_2 backpulsing technique for improving the flux in cold MF of apple cider

CHAPTER 3

EFFICIENT REDUCTION OF PATHOGENIC AND SPOILAGE MICROORGANISMS FROM APPLE CIDER BY COMBINING MICROFILTRATION WITH ULTRAVIOLET TREATMENT

Abstract

Thermal pasteurization can achieve the FDA required 5-log reduction of pathogenic *Escherichia coli* O157:H7 and *Cryptosporidium parvum* in apple juice and cider, but it can also negatively affect the nutritional and organoleptic properties of the treated products. In addition, thermal pasteurization is only marginally effective against the acidophilic, thermophilic and spore forming bacteria *Alicyclobacillus* spp., which is known to cause off flavors in juice products. In this study, the efficiency of a combined microfiltration (MF) and ultraviolet (UV) process as a non-thermal treatment for the reduction of pathogenic and non-pathogenic *E. coli*, *C. parvum* and *A. acidoterrestris* from apple cider was investigated. MF was used to physically remove suspended solids and microorganisms from apple cider, thus enhancing the effectiveness of UV, and allowing a lower UV dose to be used. MF using 0.8 µm and 1.4 µm pore size ceramic membranes was performed at a temperature of 10 °C and a transmembrane pressure of 155 kPa. The subsequent UV treatment was conducted using at a low UV dose of 1.75 mJ/cm^2. The combined MF and UV achieved more than 5 log reduction of *E. coli*, *C. parvum* and *A. acidoterrestris*. MF with 0.8 µm pore size performed better than 1.4 µm pore size on removal of *E. coli* and *A. acidoterrestris*. The developed non-thermal hurdle treatment has the potential to

significantly reduce pathogens, as well as spores, yeasts, molds and protozoa in apple cider, and thus help juice processors improve the safety and quality of their products.

Introduction

Consumption of raw apple juice or cider contaminated with *Escherichia coli* O157:H7 and *Cryptosporidium parvum* has resulted in several outbreaks and has raised public health concerns since the 1990s (Besser et al., 1993; Centers for Disease Control and Prevention (CDC), 1997; Cody et al., 1999; Diallo, Bradley, Crutcher, Lytle, Lee, & Moolenaar, 2011; Hilborn et al., 2000; Millard et al., 1994; Vojdani, Beuchat, & Tauxe, 2008). To address these safety concerns, the U.S. Food and Drug Administration (FDA) mandates in its Juice HACCP regulations that a 5-log reduction of the pertinent pathogens (*Escherichia coli* O157:H7 and *Cryptosporidium parvum*) must be achieved during manufacturing of apple juice and cider (Federal Register, 2001). Conventional thermal pasteurization usually conducted at 71.7°C for 6 s can achieve a 5-log reduction of *E. coli* O157:H7 and *C. parvum* (Mak, Ingham, & Ingham, 2001; Deng & Cliver, 2001), but it can negatively affect the nutritional and organoleptic properties of juice and cider. In addition, conventional thermal pasteurization of juices is unable to eliminate acidophilic, thermophilic and spore forming spoilage bacteria from the *Alicyclobacillus* spp. *Alicyclobacillus acidoterrestris*, in particular, has been identified to be the cause of off-flavor described as "bandage like" due to the production of guaiacol, in shelf stable apple juice products (Chang & Kang, 2004). Spores of *A. acidoterrestris* are capable of growing in low pH juices and survive thermal treatment. One study has reported a D-value for this organism of 23 min at 90 °C in apple juice (Splittstoesser, Churey, & Lee, 1994). Thermal

treatment of juice at such high temperature and long time is detrimental to the sensory properties and nutritional value of the product, and thus not used by commercial processors.

Ultraviolet (UV) treatment is a well-established, FDA-approved alternative to thermal pasteurization of apple juice and cider, which has been demonstrated to achieve a 5-log reduction of *E.* coli O157:H7 and *C. parvum* (Basaran, Quintero-Ramos, Moake, Churey, & Worobo, 2004; Quintero-Ramos, Churey, Hartman, Barnard, & Worobo, 2004; Hanes et al., 2002). Nonetheless, UV treatment is not very effective against *Alicyclobacillus* spores. A recent study showed that only about 2-log reduction of *Alicyclobacillus* spores was achieved using the FDA mandated UV dose of 14 mJ/cm^2 (Handan, Celenk, & Sevcan, 2013). High doses of UV can also negatively affect juice quality. It was shown before that UV doses larger than 10.62 J/cm^2 in a rising film UV reactor had negative effects on juice color and flavor (Caminiti et al., 2012). Moreover, the absorption and scattering of UV light by suspended solids in apple cider diminish the efficiency of UV treatment. In addition, yeasts and molds are not affected by UV treatment, which leads to a shorter shelf life of the UV treated juice and cider than of the thermally treated products, usually 2 weeks (Azhuvalappil, Fan, Geveke, & Zhang, 2010; Tandon, Worobo, Churey, & Padilla-Zakour, 2003).

Microfiltration (MF) is a membrane separation process that can be used to physically remove suspended solids and microbial contaminants from apple cider that are larger than the membrane pore size. Consequently, MF reduces the turbidity of apple cider, thus when used before UV treatment, it can enhance the effectiveness of UV and allow a lower UV dose to be used. MF with pore sizes of 0.2 µm is used by some juice, beer or wine processors and is known as sterilization microfiltration. Several studies have shown that juice MF with this pore size can be considered commercially sterile (Su, Liu, & Wiley, 1993; Fukumoto, Delaquis, & Girard,

1998). However, MF using such small pore sizes can also retain low and medium molecular weight components such as pectin and color components in the juice, thus stripping the final product of some of its most desirable properties. Therefore, using membrane pore sizes larger than 0.2 µm for microbial removal could offer the advantage of an increased retention of the juice nutritional, color and flavor components. At the same time, this could potentially lead to higher permeate fluxes compared to 0.2 µm MF, increasing the processing yield. Nonetheless, very little data exists in literature regarding the microbial removal efficiency of MF with pore sizes larger than 0.2 µm.

In this study, a hurdle technology consisting of a combination of large pore, low temperature MF and UV was developed as a non-thermal method to enhance the microbial safety of partially clarified apple cider, while preserving its shelf life and nutritional quality. The effectiveness of large pore, cold MF and the combined MF + UV treatment for the reduction of *E. coli*, *C. parvum,* and *A. acidoterrestris* from apple cider was evaluated.

Materials and Methods

Apple cider (used synonymously with apple juice): Commercially pasteurized apple cider was obtained from Cornell Dairy Operation (Ithaca, NY) and stored at 4 °C until use. Due to the seasonal production, some batches of pasteurized cider were stored frozen at -20 °C and thawed at 4 °C before use. The cider was inoculated with a pure culture of *E. coli* (both pathogenic and non-pathogenic strains), *C. parvum* or *A. acidoterrestris* immediately prior to the hurdle treatment.

Physicochemical analyses of cider and juice

pH was measured using an Accumet Basic AB15 pH meter (Fisher Scientific, Pittsburgh, PA) and °*Brix* was measured using a Leica Auto Abbe refractometer model 10500-802 (Leica Inc., Buffalo, NY). *Total titratable acidity* was measured using a G20 compact titrator (Mettler Toledo, Schwerzenbach, Switzerland). *Turbidity* (in NTU) was measured using a HACH 2100P portable turbidimeter (Hach Company, Loveland, CO). *Lab color* was measured using a Hunter UltraScan XE spectrocolorimeter (Hunter Lab Assoc., Reston, VA).

Culture and inoculum preparation and microbiological analyses

E. coli: E. coli ATCC 25922, often used as a non-pathogenic surrogate for *E. coli* O157:H7, and a 5 strain cocktail of pathogenic *E. coli* O157:H7 (a cocktail of five pathogenic strains in equal proportion: 43889, C7927, 43894, 43895 and 35150) were obtained from Dr. Randy Worobo's laboratory in the Department of Food Science at Cornell University (Geneva Experiment Station, NY). These cultures were stored on Trypticase Soy Agar (TSA; Difco, Becton Dickinson, Franklin Lakes, NJ) plates at 4 °C for up to 1 month. Once a month, a colony was transferred to a new TSA plate. One day before use, a single isolated *E. coli* colony was transferred into 10 ml of Trypticase soy broth (TSB; Difco, Becton Dickinson, Sparks, MD) and incubated for 5 ± 1 h at 35 ± 2 °C on a rotary platform shaker at 250 rpm. The inoculated TSB was then transferred into 400 ml of TSB and incubated for 20 ± 2 h at 35 ± 2 °C to stationary phase on a rotary platform shaker at 250 rpm.

Inoculation of approximately 14 L of pasteurized apple cider with *E. coli* ATCC 25922 and *E. coli* O157:H7 5-strain cocktail at a level of 10^6 to 10^7 CFU/ mL was done immediately before processing, to avoid acid injury. Samples of the product before MF treatment (control), after MF treatment and after UV treatment were collected aseptically and analyzed immediately by Standard Plate Counting. Appropriate serial dilutions (1ml) in 0.1% peptone (Difco, Becton

Dickinson, Franklin Lakes, NJ) were pour plated in duplicate on plate count agar (PCA; Acumedia Manufactuer, Inc., Baltimore, MD) and incubated at 37°C for 24 h. Plates were counted and results expressed in Log CFU (colony forming units). Experiments were conducted in triplicate.

C. parvum: A suspension of *C. parvum* oocysts of known concentration (10^9 oocysts/ mL) dispersed in a phosphate-buffered saline solution containing penicillin, streptomycin, gentamicin, amphotericin B and 0.01% Tween 20 was purchased from Waterborne Inc. (New Orleans, LA). The oocyst suspension was stored at 4 °C and used within 2 months. Inoculation of approximately 14 L of pasteurized apple cider with *C. parvum*, at a level of 10^6 to 10^7 oocysts /ml was performed immediately before the MF and UV hurdle treatment, to avoid acid injury of oocysts. Samples of untreated, inoculated cider (control) and samples after the MF treatment and after the combination MF+UV treatment were collected aseptically. Experiments were conducted in duplicate.

The cider / juice samples were centrifuged at 931 x g for 10 min to pellet oocysts and the pellets were then re-suspended in PBS. This liquid was used in cell culture and analyzed by a cell culture infectivity assay using a human ileocecal cell line (HCT-8). Quantification was done by DNA extraction coupled with PCR for the sporozoite heat shock and oocyst wall proteins. Cell cultures were maintained and infected with treated or untreated *C. parvum* oocysts. Infectivity of *C. parvum* oocysts was determined by quantitative RT-PCR (Johnson, Giovanni, & Rochelle, 2012).

In vitro cell culturing of C. parvum and oocyst treatment: Human ileocecal adenocarcinoma (HCT-8; ATCC CCL-244) was maintained in RPMI 1640 medium (Mediatech Cellgro, Herndon, VA) supplemented with L-glutamine (300 mg/L; Mediatech Cellgro), HEPES

(25mM; Mediatech Cellgro), and fetal bovine serum (pH 7.2; Biofluids, Inc., Rockville, MD). For normal cell maintenance medium was supplemented with 10% fetal bovine serum and decreased to 5% fetal bovine serum for parasite infection. HCT-8 cells were cultivated in 6-well cell culture plates and incubated at 37°C in a 5% CO_2 humidified incubator to allow the development of 85 to 90 % confluence in medium for 48 h. The cell monolayer in each well of the plate was considered a single replicate.

Inoculation of monolayers with oocysts: A modified version of methods previously published is described below (Najdrowski, Joachim, & Daugschies, 2007). Prior to infection of oocysts on the cell monolayer, the cell growth media was removed and cells were washed with HBSS to remove any cellular debris. Samples of control oocysts or treated oocysts (1 mL) were added to wells of culture plates in duplicate. The plates were placed in the incubator at 37 °C for 1 h to allow for infection of the cells. The inoculum was then removed, and 2 mL of fresh media was added in each well. The plates were placed in the incubator at 37 °C with 5% CO_2 for 48 h for replication. Media was removed, and each well was rinsed by 1 mL HBSS 3 times. Five hundred µL accutase (BD Biosciences, San Jose, CA) was added in each well and plates were incubated for 10 min at 37 °C or until cell layer became detached as determined by microscopy. Media, HBSS, as well as detached cells were collected for each well for DNA extraction.

DNA extraction from oocysts and cell culture monolayers: DNA extraction was performed using QIAmp DNA Mini Kit (Qiagen, Hilden, Germany). In the last step the DNA were eluted from the column with 20 µL elution buffer and stored at 4 °C for a maximum of 1 week until PCR amplification.

Real-time PCR Quantification: Real-time PCR quantification was performed using SYBR green fluorescence. PCR primers specific for the *C. parvum* heat shock protein gene

(hsp70) were designed as follows: forward primer (5'-TTGAACGTATGGTTAATGATGCT-3') and reverse primer (5'-CGAGCCAGTCAAGAGCATCC-3'). Each 20 µL reaction contained 10 µL of SYBR Master Mix (QIAGEN, Valencia, CA), 0.6 µL of forward and reverse stock of 10 µM (final concentration is 0.3 µM), 0.8µL sterile RNase DNase free water, and 8.0 µL of DNA template. Amplification conditions were as follows: initial denaturation at 95 °C for 10 min, and 40 cycles of denaturation at 94 °C for 10 s, annealing at 60 °C for 20 s, and extension at 72 °C for 45 s, followed by a dissociation step at 60 °C for 15 s and 90 °C for 15 s. Each sample was analyzed in duplicate.

Purified DNA that was extracted from log 6 *C. parvum* oocysts was serially diluted from log 6 oocysts/mL down to log 1 oocyst/mL to create a standard curve. RNase DNase free water replacing template DNA served as a negative control. The *C. parvum* standard curve obtained was used to determine log number of oocysts of each sample on the basis of the Ct values.

A. acidoterrestris VF (vegetative cells and spores)*:* Isolates of *A. acidoterrestris* VF strain were obtained from Dr. Randy Worobo's laboratory in the Department of Food Science at Cornell University (Geneva Experiment Station, NY) and grown on acidified Potato Dextrose Agar (APDA, pH 3.5, Becton Dickinson, Franklin Lakes, NJ) at 50 °C for 7 days to reach stationary phase before inoculation. Spores were harvested by adding 1-2 mL of sterile deionized water onto the APDA surface and gently rubbing the media surface using a sterile cotton swap. This rinsing procedure was repeated for three times and suspensions were pooled and centrifuged at 4000 x g for 20 min at 4 °C. The supernatant was discarded and the precipitate was re-suspended in sterile deionized water, then vortexed and centrifuged again. This washing procedure was repeated three times.

Inoculation of approximately 14 L of pasteurized apple cider with *A. acidoterrestris* VF and spores, at a target level of 10^6 CFU/ml, was performed immediately before processing. Samples of untreated, inoculated cider (control) and samples after the MF treatment and after the combination MF and UV treatment were collected aseptically and analyzed immediately. Cell count was determined by preparing serial dilutions of each sample using 0.1% of peptone water (Difco, Becton Dickinson, Franklin Lakes, NJ). Appropriate serial dilutions (1 mL) were plated in duplicate on APDA (pH 3.5, Becton Dickinson, Franklin Lakes, NJ) and incubated at 50 °C for 96 h. All plate counts were averaged and expressed as log CFU. To determine the total cell and spore counts, collected samples were heat shocked at 80 °C for 10 min to induce the spores to germinate, followed by the cell counting procedure as outlined above. Spore counts were equal to the total cell and spores counts minus the cell counts. All spore counts were averaged and expressed as log number. Experiments were conducted in triplicate.

Cell size and Zeta potential measurements

Cultures of *E. coli* ATCC 25922 and *E. coli* O157:H7 (a cocktail of five strains: ATCC 43894, ATCC 43895, ATCC 35150, ATCC 933, and ATCC 43889) and *A. acidoterrestris* VF were cultured as described above. All strains were harvested at stationary phase by centrifugation at 2767 x g using a Hettich 32R benchtop centrifuge (Hettich Instruments, Beverly, MA). To remove media residues from the cell suspension, the cells were re-suspended by vortexing in 30 mL of Butterfield's phosphate buffer (BPB) (pH 7.0 ± 0.2, ionic strength 0.01 M) and then re-collected by centrifugation (at 4 °C, 2767 x g, 10 min). Finally, the cells were re-suspended again in BPB, and used for particle size and zeta potential analyses.

Zeta potential. The zeta potential of bacterial cells, used as an indicator of their surface charge, was measured using a Malvern Zetasizer nano-ZS (Malvern Instruments Ltd., Malvern,

Worcestershire, United Kingdom) with disposable folded capillary cells (Malvern Instruments Ltd., Malvern, Worcestershire, United Kingdom). Briefly, a 1:10 dilution of cells (prepared as described above) was prepared in pH 3.5 phosphate buffer (ionic strength 0.5 M, representative of apple juice) and gently vortexed to ensure thorough mixing. A 1 mL sample was aliquoted into the cuvette, which was then inserted into the measurement chamber. Zeta potentials were measured at 20 °C, in triplicate, using 100 cycles per analysis for each of the biological triplicates.

To measure the zeta potential of the material for the ceramic membrane used in this study, 2 g of ceramic membrane was ground into a fine powder using a pestle and mortar. The powder was then suspended into 10 mL deionized water (Mili-Q; Merck Millipore Ltd., Billerica, MA), vortexed thoroughly, and allowed to settle for 40 min without disturbance. Next, 0.1 mL of the supernatant was transferred into 0.9 mL of pH 3.5 buffer to make a 1:10 dilution. A 1 mL sample from the resulting suspension was aliquoted into a cuvette, and zeta potential were measured at 20 °C, in triplicate, using 100 cycles per analysis for each of the triplicates.

Cell size measurement. The cell sizes of the bacterial cultures were also measured using the Malvern Zetasizer nano-ZS following the same dilution scheme used for bacteria zeta potential measurements. One mL of the diluted sample was aliquoted into a cuvette (UV-cuvette semi-micro, Brandtech, Essex, CT), which was then inserted into the measurement chamber. The measurement was carried out at 20 °C, and was repeated at least twice for each of the biological triplicates. The intensity mean of cell diameter was used to represent cell size in this study.

Processing methods: MF and UV

Inoculated cider samples were first subjected to MF, followed immediately by the UV treatment of the MF permeate.

Microfiltration (MF) treatment: The pilot-scale MF unit consisted of a feed tank connected to a variable-speed centrifugal pump, a tubular heat exchanger and a ISOFLUX™ tubular ceramic membrane of TAMI design (GEA Filtration, WI) placed inside a stainless steel housing. The membrane had an outside diameter of 25 mm, length of 1200 mm, 23 internal channels of 3.5 mm hydraulic diameter each, and a membrane area of 0.35 m^2. Two membranes with various pore sizes were used in this study: 0.8 μm and 1.4 μm. The cold MF of raw apple cider was conducted at a temperature of 10 ± 1°C. This temperature was chosen to preserve the quality of apple cider and juice and minimize microbial growth during processing.

Feed inlet pressure (P_1), retentate outlet pressure (P_2), and permeate pressure (P_p) were recorded and the transmembrane pressure (TMP), calculated using equation (1):

$$TMP = \frac{(P_1 + P_2)}{2} - P_p \qquad (1)$$

All pressures were expressed in kPa. The permeate pressure (P_p) equaled the atmospheric pressure. The transmembrane pressure in all MF runs was 155 kPa.

Membrane cleaning: After each MF experiment, 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 (Ecolab, St. Paul, MN) at a concentration of 20 g/L at 80 °C for 30 min and a second Reverse Osmosis (RO) water rinse for 10 min or until neutrality. Acid cleaning with 5 mL/L HNO_3 at 50 °C for 20 min was then performed, followed by a third RO water rinse for 10 min or until neutrality. A sterilization step consisting of heating the entire MF system at 80 °C for 30 min for the *E. coli* and *C. parvum* experiments and 95 °C for 30 min for the *A. acidoterrestris* VF was conducted prior to each use. The effectiveness of

cleaning and change in the membrane performance with time were monitored by determining the water flux before and after the MF runs.

UV treatment: UV-C irradiation of the permeate samples from the MF treatment was carried out using a CiderSure UV-C unit model 3500 (FPE Inc., Macedon, NY). The UV reactor comprises a stainless steel outer housing and an inner quartz tube. The UV light exposure of the fluid, at a dose of 1.75 mJ/cm^2, was provided by one germicidal low-pressure mercury lamp placed inside the quartz-stainless steel cylinder. The CiderSure UV-C unit model 3500 was equipped with a sensor system, which can adjust the flow rate of the system to ensure that the targeted dose was delivered to the liquid.

Samples of inoculated cider (control), juice after MF treatment (permeate) and after the MF+UV treatment (UV treated permeate) were collected and analyzed.

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.

Results and Discussion

The physicochemical characteristics of pasteurized apple cider before inoculation with *E. coli*, *C. parvum* and *A. acidoterrestris* VF were measured and values are reported in Table 1. After MF, clear apple juice was obtained. MF was found to have no significant effect on the pH ($p_{0.8}$=0.6327 and $p_{1.4}$=0.8109), °Brix ($p_{0.8}$=0.1491 and $p_{1.4}$=0.2359), and TA (%) ($p_{0.8}$=0.4270 and $p_{1.4}$=0.0862) of the product (Zhao and Moraru unpublished data) and UV was proven to have no effect on apple juice pH and °Brix (Caminiti et al., 2012). Due to biological safety concerns, inoculated samples were not subjected to physicochemical measurements.

Table 1. **Physicochemical characteristics of pasteurized apple cider used as feed in the microbial challenge studies. Values represent means ± SD, n=2.**

Challenge study	pH	Soluble Solids (°Brix)	Titratable Acidity (% malic acid)	Turbidity (NTU)	Color parameters		
					L	a	b
E. coli ATC25922, MF/0.8 µm	3.72±0.04	12.75±0.38	0.35±0.03	556±20	29.91±0.33	1.09±0.13	2.58±0.25
E. coli O157:H7, MF/0.8 µm	3.79±0.02	14.35±0.96	0.42±0.02	815±190	34.54±0.49	5.13±1.29	10.06±1.09
E. coli ATC25922, MF/1.4 µm	3.74±0.03	13.05±0.39	0.34±0.04	549±30	29.77±0.40	0.82±0.08	2.32±0.19
E. coli O157:H7, MF/1.4 µm	3.73±0.06	13.30±0.10	0.33±0.04	525±28	29.83±0.19	0.83±0.08	2.42±0.11
C. parvum, MF/0.8µm	3.66±0.07	15.07±0.05	0.48±0.02	1136±189	33.37±0.20	2.02±0.07	7.65±0.24
C. parvum, MF/1.4µm	3.66±0.04	13.08±1.02	0.41±0.05	493±236	32.30±0.26	1.45±0.03	6.94±0.23
A. acidoterrestris VF, MF/0.8µm	3.62±0.03	13.34±3.15	0.43±0.04	1041±98	42.57±1.73	10.00±0.74	25.20±1.24
A. acidoterrestris VF, MF/1.4µm	3.71±0.05	14.35±0.09	0.44±0.02	787±65	43.86±0.25	10.49±0.05	27.25±0.45

MF using a 0.8 µm membrane pore size achieved more than 7-log reduction of the non-pathogenic *E. coli*, and 5-log reduction of the pathogenic *E. coli* O157:H7. When using a 1.4 µm pore size, MF achieved 4.7-log reduction of the non-pathogenic *E. coli* and 4.6-log reduction of the pathogenic *E. coli* O157:H7. For both pore sizes, the combined MF and UV process achieved a greater than 7-log reduction of both *E. coli* strains (Table 2).

Table 2. Microbial reduction of non-pathogenic and pathogenic *E. coli* by combined MF (0.8 and 1.4 µm) and UV treatment (at a dose of 1.75 mJ/cm^2). Values represent means ± SD, n=2.

Challenge study	Initial load (log CFU/mL)	After MF/before UV (log CFU/mL)	After MF+UV (log CFU/mL)
E. coli ATCC 25922, MF/0.8µm	7.89±0.17	0.53±0.08 *(MF reduction: 7.36log)*	n.d.*
E. coli O157:H7, MF/0.8µm	7.68±0.35	2.51±0.57 *(MF reduction: 5.17log)*	n.d.*
E. coli ATCC 25922 , MF/1.4µm	7.95±0.22	3.27±0.17 *(MF reduction: 4.68log)*	n.d.*
E. coli O157:H7, MF/1.4µm	7.67±0.09	2.74±0.71 *(MF reduction: 4.93log)*	n.d.*

**Below detection limit of 1CFU/mL*

A complete removal of infectious *C. parvum* oocysts was achieved, regardless of the membrane pore size. No infectious oocysts were found in juice after the combined MF and UV treatment (Table 3).

Table 3. Reduction of *C. parvum* oocysts using MF and UV (at a dose of 1.75 mJ/cm^2). Values represent means ± SD, n=2.

Method of analysis	Initial load* (control)	After MF*/ (MF Permeate)	MF Retentate*	After MF* + UV
Detection (+/-)	+	-	+	-
Quantification (log oocysts/mL)	5.30 ± 1.08	n. d.** (complete removal)	4.89 ± 0.15	n. d.**

** Results apply to both membrane pore sizes (0.8 µm and 1.4 µm)*

*** Detection limit: 10^2 oocysts/mL*

MF using a 0.8 µm pore size membrane achieved more than 5-log reduction of the spoilage bacterium *A. acidoterrestris* VF (vegetative cells and spores), while MF using 1.4 µm achieved more than 4.8 log reduction of vegetative cells and spores. After the combined MF (using both pore sizes) and UV (at a low dose of 1.75 mJ/cm^2), more than 5-log reduction of *A. acidoterrestris* VF (cell and spores) was achieved (**Table 4**).

Table 4. Reduction of *A. acidoterrestris* VF cells and spores by combined MF (0.8 and 1.4 µm) and UV treatment (at a dose of 1.75 mJ/cm^2). Values represent means ± SD, n=2.

MF pore size	A. acidoterrestris VF	Initial load (log CFU/mL)	After MF (log CFU/mL)	After MF+UV (log CFU/mL)
0.8 µm	Cells	5.61±0.10	n.d.* (MF reduction > 5 log)	n.d.*
	Spores	5.70±0.06	n.d.* (MF reduction > 5 log)	n.d.*
1.4 µm	Cells	5.67±0.11	0.86±0.99** (MF reduction: 4.81 log)	n.d.*
	Spores	5.70±0.11	0.45±0.39** (MF reduction: 5.25 log)	n.d.*

**Below detection limit*

***Some replicates were below detection limit*

These results were generally expected, but the degree of physical removal of the bacterial cells by MF warrants further discussion. As seen in Table 5, the average cell size for the *E. coli* cells was very close to the nominal pore size of the 0.8 µm MF membrane, but significantly smaller than the pore size of the 1.4 µm MF membrane. Based on size alone, one would have expected a complete removal of *E. coli* by the smaller size membrane and complete passage through the larger pore size membrane, which is different than what was observed. First, the 0.8 µm MF membrane still allowed the passage of some cells. It must be noted though that the particle size values reported in Table 5 are diameters of equivalent spheres (spheres that have the same volume as the cells). This assumes the bacterial cells to be circular, whereas *E. coli* cells are rod shaped, and are known to measure approximately 0.5 µm in width and 2 µm in length.

The size of rod shaped *A. acidoterrestris* from soil and apple juice has been reported to be 2.9 to 4.3 µm in length and 0.6 to 0.8 µm in width, and the oval shaped spores were reported to measure 1.5 to 1.8 µm in length and 0.9 to 1.0 µm in width (Wisotzkey, Jurtshuk, Fox, Deinhard, & Poralla, 1992; Walls & Chuyate, 1998). Therefore, it was possible for some of the cells to penetrate through the 0.8 µm pore size membrane, if they were oriented perpendicular to the membrane. This shows also the need for the final UV kill step after MF with a pore size larger than 0.2 µm.

Table 5. Average size and zeta potential of bacterial strains and the MF ceramic membrane used in the study

Sample	Zeta Potential (mV)	Diameter (nm)
Ceramic Powder	-8.53 ± 1.18	-
Bacteria		
E. coli O157:H7 (5 strain cocktail)	-2.08 ± 0.51	734 ± 43
E. coli ATCC 25922	-11.31 ± 1.59	739 ± 161
A. acidoterrestris VF	-5.40 ± 0.71	623 ± 41

** All values were measured in phosphate buffer (pH=3.5, ionic strength =0.5, temperature = 20 °C)*

The data in Table 5 is however very useful when comparing directly the relative size of the different bacterial strains. The particle size data indicates for instance that the cells for the pathogenic and non-pathogenic *E. coli* strains are similar in size, yet the reduction data in Table 2 indicates significant differences (p=0.0264) between the pathogenic and non-pathogenic strains in the reduction using the 0.8 µm pore size MF membrane. An explanation for this difference can

be offered by the difference in surface charge of these bacterial strains. As seen in Table 5, zeta potential values at the pH and ionic strength similar to apple cider are negative for both the membrane material and the bacterial cells. This indicates an electrostatic repulsion between the membrane material and the bacterial cells. Yet, this repulsion is likely much stronger for the *E. coli* ATCC 25922 (ζ = -11.31 mV) as compared to *E. coli* O157:H7 (ζ = -2.08 mV), due to the larger magnitude of the zeta potential of the former. This means that *E. coli* ATCC 25922 cells will be stronger repelled by the ceramic membrane material, and thus are less likely to penetrate the membrane (at the same shape and size) as compared to *E. coli* O157:H7. The implication is that *E. coli* ATCC 25922 is not a suitable non-pathogenic surrogate for *E. coli* O157:H7 in case of MF of apple cider using ceramic membranes, despite their similar size. This is very important to note, since *E. coli* ATCC 25922 is often used as a surrogate for *E. coli* O157:H7, but this data suggests that in this particular situation this is not the case. Nonetheless, this conclusion should not be generalized to all membrane filtration situations, since surface charge is affected by pH and ionic strength, and the situation may be different for other fluids. Rather, our recommendation is that fully characterizing the surface properties of the membrane material and microbial cells should be used as a base for determining suitable surrogates for pathogenic organisms in each case for this technology.

The bacterial removal efficiency of the 1.4 μm membranes also needs to be discussed. As discussed above, the length of the *E. coli* and *A. acidoterrestris* rods can exceed slightly the 1.4 μm pore size, which is in part the reason why some of the cells are retained by the membrane. Another reason is likely to be fouling of the membrane by some of the rejected cider solids. During MF, suspended particles from the feed are known to cause pore constriction, pore blocking and cake layer formation on the membrane surface. This is generically termed

membrane fouling, and can reduce the effective membrane pore size. Therefore, the effective pore size of the membranes during the MF process was most likely smaller than the nominal pore size declared by the manufacturer, which helped retain more bacterial cells than what would have been expected based on nominal size.

For *C. parvum* oocysts, which have a diameter of approximately 5 µm, MF using both 0.8 and 1.4 µm was able to achieve complete removal of the oocysts. The presence of viable stages (+) of *C. parvum* oocysts in the MF retentate (Table 3) indicates that the oocysts maintained their viability during MF, and thus the fact that they were not detected in the permeate was due to physical removal and not injury during MF. Nonetheless, some mechanical damage of the oocysts may have occurred due to shear forces generated during the MF process, as suggested by the lower concentration in retentate as compared to the feed (control).

Using MF before the UV treatment removed some of the suspended solids from cider, including yeast and molds, allowed a low UV dose used in this study, 1.75 mJ/cm^2, which is about 1/8 of the FDA recommended dose for treatment for apple cider The applied UV treatment was able to act as a final kill step and the combined MF and UV process achieved more than 5-log reduction of both the non-pathogenic and pathogenic *E. coli* and of the spoilage bacteria *A. acidoterrestris* (vegetative cells and spores). The UV treatment is only a finishing step, which ensures that a safe process will be achieved even if there is some passage of microorganisms due to their size, shape and orientation relative to the membrane or even due to a microscopic internal defect in the membrane structure. The data also suggested that the UV dose could be further reduced, which could lead to energy savings and / or a speeding of the process.

Overall, this study demonstrates that the combined MF and UV process can be effective for the reduction of *E. coli* O157:H7 and *C. parvum* in apple cider and meet the 5-log reduction

required by the Juice HACCP regulation. In addition, the efficient removal of *A. acidoterrestris* by the non-thermal hurdle process developed here provides a feasible solution to extend the shelf life of apple cider and juice products. This hurdle technology has great potential as a non-thermal alternative to heat pasteurization and ensuring the safety and quality of apple cider and juice, and potentially of other juice products.

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CHAPTER 4

INVESTIGATION OF MF FOULING MECHANISMS AND PROCESS OPTIMIZATION

4.1 OPTIMIZATION OF LARGE PORE MICROFILTRATION OF APPLE CIDER

Abstract

Microfiltration (MF) process conditions, such as cross-flow velocity and transmembrane pressure (TMP), and their effect on the efficiency of the process were investigated for the cold MF of apple cider using a 0.8 μm pore size membrane. It was found that the highest flux and the lowest flux decline were achieved at a cross-flow velocity of 5.5 m/s and TMP of 159 kPa. After MF, clear apple juice was obtained. The change in color and a decrease in turbidity after MF were due to the removal of suspended insoluble solids in the apple cider. This optimization study was used as a base for subsequent work involving cold MF of apple cider.

Introduction

Microfiltration (MF) has been used by the juice and beverage industry for many years, primarily for clarification, as an alternative to the conventional fining and filtration methods, including dead end filtration or diatomaceous earth filtration. MF is a pressure-driven process that can physically remove suspended solids that are larger than the effective membrane pore size. The benefits of using this technology include that fact that it is a continuous operation, which does not require filtration aids, and that the membranes have the ability of regeneration. For juice applications, besides achieving a clarifying effect, MF can remove many of the

pathogenic and spoilage microorganisms or concern, including bacteria, yeast, mold and protozoa.

The major challenge that still limits the use of MF by the juice industry is the significant decline in permeate flux over time due to membrane fouling. In order for MF to be widely adopted at an industrial scale, it is very important to find ways to reduce fouling. Many factors can affect fouling and the permeate flux, namely processing conditions, membrane characteristics and properties, the physicochemical properties of the feed material and the interactions between the membrane material and feed components.

One strategy is to optimize the process parameters. MF process conditions that affect fouling include temperature, cross flow velocity, and transmembrane pressure (TMP), and these are usually optimized for a given MF application (Fritsch & Moraru, 2008). Temperature has a positive effect on the permeate flux, due to a decrease of viscosity of feed and increased back diffusion of the foulants into the feed stream at higher temperatures (Padilla-Zakour & McLellan, 1993). MF of apple cider is typically carried out at 50-55°C (Girard & Fukumoto, 1999; Rao, Acree, Cooley, & Ennis, 1987). Nonetheless, such temperatures can facilitate the growth of thermophillic bacteria in the membrane system, which could cause an increase of microbial load in the feed and even accelerate membrane fouling. Running MF at low temperatures (6-10°C) could avoid this problem. Cross-flow velocity also has a positive effect on the permeate flux. High cross-flow velocities create a turbulent flow, which can minimize the deposition of foulants onto the membrane. The shear forces created by high cross-flow velocities also enhance the hydrodynamic diffusion and reduce fouling formation, thus improving the permeate flux (Moraru & Schrader, 2008). The effect of TMP on flux and fouling is more complex and has often been explained using the critical flux concept (Field, Wu, Howell, & Gupta, 1995; Howell, 1995).

According to the critical flux theory, when a MF system is operated below the critical flux (regime I), no fouling layer forms on the membrane surface and the permeate flux increases with TMP. Regime II represents a region where flux is independent of TMP; under this regime, particle deposition onto the membrane surface is equilibrated by the back transport of suspended particles into the feed stream due to hydrodynamics. When TMP exceeds a critical value (regime III), an irreversible cake layer forms and further increase of TMP causes compaction of the cake layer and a significant increase of fouling resistance (Figure 1). For clarification purposes, operating in regime II is desirable since it gives the best permeability and highest MF capacity. It is also important to note that TMP and cross-flow velocity are usually coupled in a MF system, which means that an increase in velocity leads to an increase in TMP.

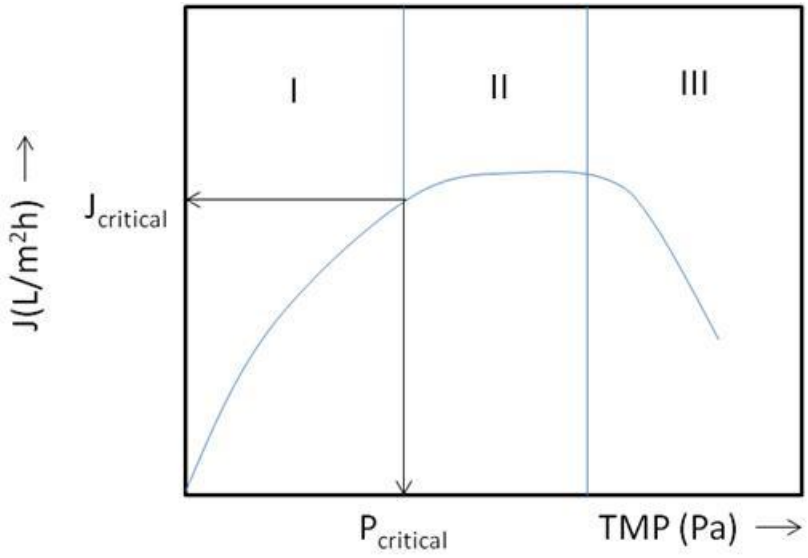

Figure 1. Dependence of permeate flux on TMP according to the critical flux theory (Brans, Schroën, van der Sman, & Boom, 2004).

The objective of this work was to optimize processing parameters in large pore MF of apple cider.

Materials and Methods

Cold, raw apple cider was obtained from Cornell Orchards (Ithaca, NY), and was stored at 4°C for a maximum two weeks before being processed.

Microfiltration of apple cider. A pilot-scale MF unit consisting of a 50 gallon 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 area of 0.35 m^2. For the purpose of optimizing MF parameters, a pore size of 0.8 μm membrane was used in this study.

The MF of raw apple cider was conducted at a temperature of 6 $\pm$ 1°C, by circulating chilled water in a counter current tubular heat exchanger. This temperature was chosen to preserve the quality of apple cider and juice and reduce browning during processing. Four different cross-flow velocities were investigated: 3.5, 4.5, 5.5 and 6.3 m/s; the corresponding levels of TMP were 73, 111, 159, and 220 kPa. The highest value of cross-flow velocity and TMP were limited by the capacity of the pump used.

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

$$TMP = \frac{(P_1 + P_2)}{2} - P_p \qquad (1)$$

where permeate pressure (P_p) is the atmospheric pressure.

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

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

where: J: permeate flux (L/m^{2}h); M: amount of permeate (L) 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). Initial flux value was taken at 7 minutes after starting the pump and final flux was taken after one hour of each run.

The relative flux was calculated as follows:

$$Relative\ flux = \frac{J}{J_o} \times 100\% \qquad (3)$$

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

The value of the relative flux relates to membrane fouling, i.e. a lower J/J_0 indicates a more pronounced fouling of the membrane than a higher J/J_0 value. This normalized parameter allows direct comparisons among MF experiments that have different permeate flux values. All MF experiments were duplicated.

Membrane cleaning. After each MF experiment, a chemical cleaning cycle was carried out. The cleaning procedure consisted of a rinse with RO water for 10 minutes, followed by alkaline cleaning with Ultrasil-25 at a concentration of 20g/L at 80 °C for 30 minutes and a second RO water rinse for 10 minutes or until neutrality. Acid cleaning with 5mL/L HNO_3 at 50 °C for 20 minutes was then performed, followed by a third RO water rinse for 10 minutes or until neutrality. The effectiveness of cleaning and change in the membrane performance with time were monitored by determining the water flux before and after the experiments.

The cider and MF juice were subjected to physic-chemical analyses, as described below.

pH and soluble solids content (°Brix) of the cider and MF juice were measured using an Accumet Basic AB15 pH meter (Fisher Scientific, Pittsburgh, PA) and a Leica Auto Abbe refractometer model 10500-802 (Leica Inc., Buffalo, NY).

Titratable acidity (TA) of apple cider and MF juice, expressed as percentage of malic acid, was determined using a G20 compact titrator (Mettler Toledo, Schwerzenbach, Switzerland).

A Hunter UltraScan XE spectrocolorimeter (Hunter Lab Assoc., Reston, VA) was used to measure the Lab **color** parameters of the apple cider and MF juice.

The **turbidity** was determined using a HACH 2100P portable turbidimeter (Hach Company, Loveland, CO) and expressed as NTU.

The **suspended insoluble solids (SIS)** content was determined by centrifuging 10 mL of apple cider sample at 2200 x g for 15 min. After discarding the supernatant, the pellet (representing the SIS) was weighed and SIS was calculated and expressed as g/L.

Statistical analysis. The experimental 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.

Results and Discussion

Under all processing conditions, the permeate flux decreased with time, which suggests the occurrence of fouling. Nonetheless, significant differences in flux and fouling rate were obtained under different cross-flow velocity and TMP conditions.

As cross-flow velocity (and implicitly TMP) increased, permeate flux increased, and the highest flux was obtained at the highest velocity (6.3 m/s) (Figure 2). Nonetheless, the rate of fouling was also increased as the cross-flow velocity increased (Figure 3). For 6.3 m/s, the relative flux after 1 h of MF was 76% (Figure 3). At a velocity of 5.5 m/s, the second highest

flux was obtained and at this condition, the relative flux after 1 h was 79%, which indicated better performance over time than MF conducted at a cross-flow velocity of 6.3 m/s.

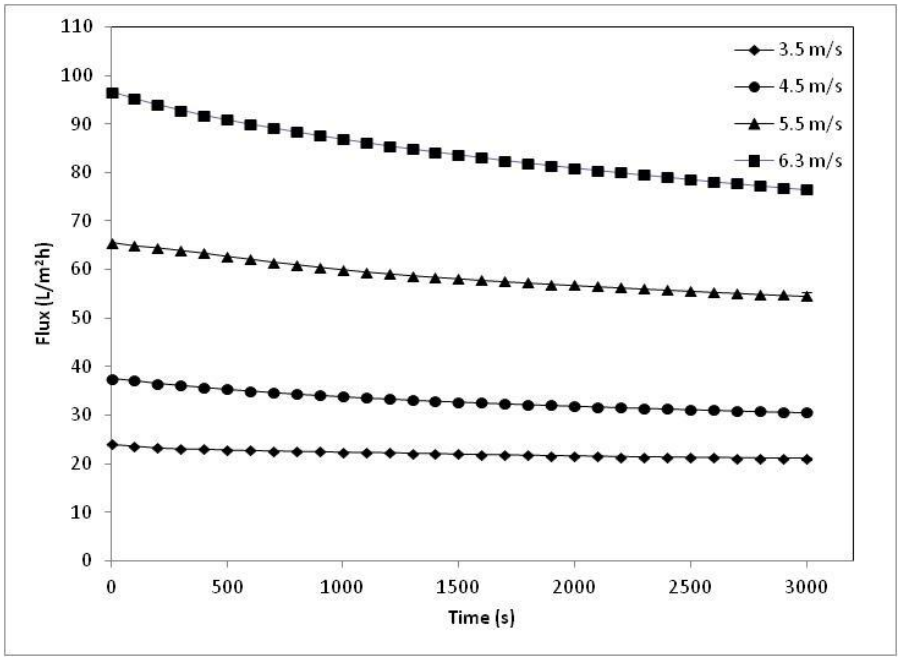

Figure 2. Permeate flux in cold MF of apple cider at different cross-flow velocities.

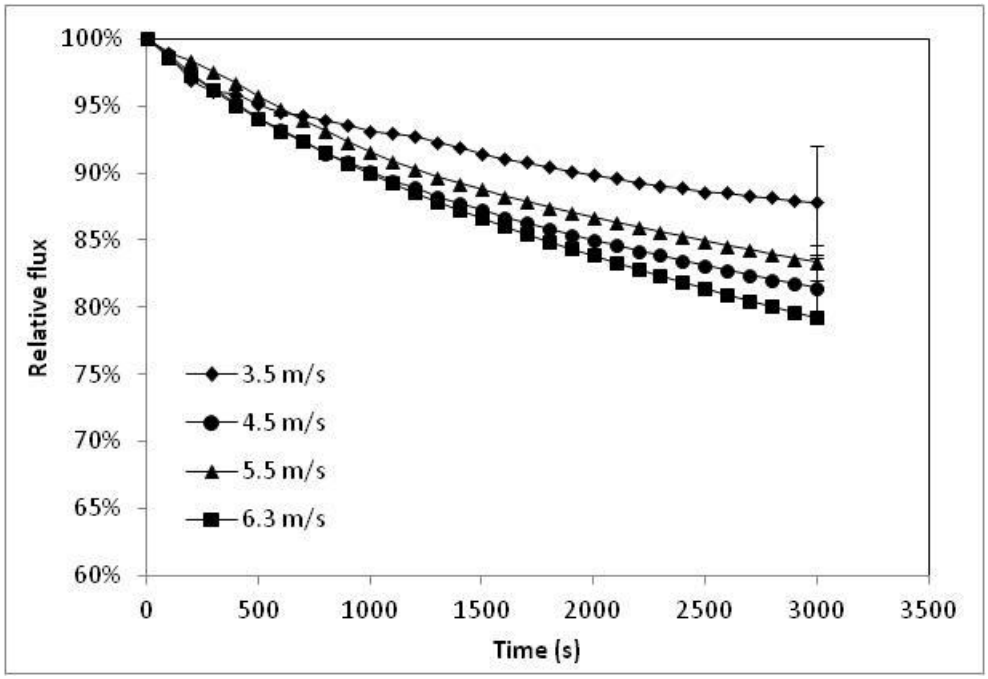

Figure 3. Relative flux at different cross-flow velocities.

This behavior can be explained using the critical flux theory: at a cross-flow velocity of 6.3 m/s (TMP of 220 kPa), the high TMP may cause irreversible fouling and compaction of fouling layer, therefore, the resistance of fouling layer increased (Regime III, Figure 1). Even if the high cross-flow velocity resulted in an initial high flux, the fouling rate was very high and thus the performance of the MF process will decline at a very fast rate, making the process inefficient.

A long term projection for a 24 hour run was conducted. The projected flux graph is shown in Figure 4, and the calculated projections, which also considered the scheduled cleaning, are shown in **Table 6**.

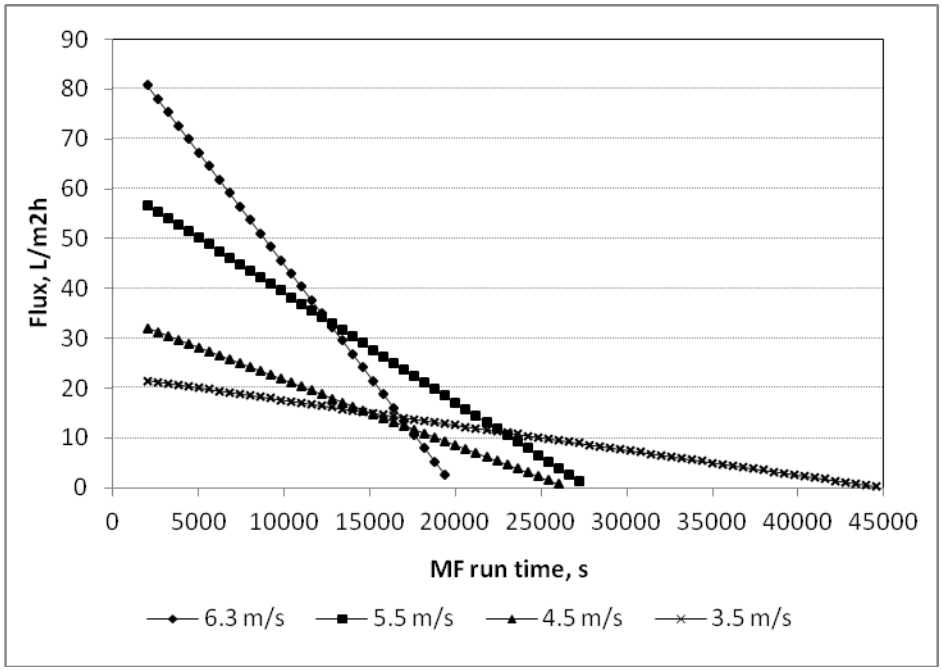

Figure 4. Long term projection of permeate flux for different cross-flow velocities.

Figure 4 clearly shows that running MF at the highest velocity result in the highest flux, but also the fastest fouling. Since this cross-flow velocity also corresponds to the highest TMP, it is likely under these MF conditions correspond to Regime III in Figure 1. The flux decreases

rapidly to zero under these conditions, which was projected to occur after 5.4 h (**Table 6**). At the other extreme, the lowest cross-flow velocity (3.5 m/s) resulted in the lowest flux, but also the least rate of fouling, and the flux was projected to reach zero after 12.4 h (**Table 6**).

Comparing the amounts of permeate projected to be collected in 24h, a velocity of 6.3 m/s resulted in the highest amount of permeate, followed closely by the 5.5 m/s, while the lowest two velocities yielded a much lower amount of permeate. However, when comparing the ratio of running time vs. cleaning time, a much more favorable ratio was obtained for 5.5 m/s (1:1) vs 6.3 m/s (0.7:1). Based on the assumptions made in these calculations, running at 6.3 m/s would require more downtime to clean the system than running at 5.5 m/s 5.5 m/s during a 24 hour cycle. Thus, taking into the consideration of energy and utility consumption, running at a velocity of 5.5 m/s (TMP of 159 kPa) was selected as the optimal condition.

Table 6. Long term projection of flux and amount permeate collected in 24 hours, including scheduled cleaning.

Velocity, m/s	**6.3 m/s**	**5.5 m/s**	**4.5 m/s**	**3.5 m/s**
Initial flux (J_0), L/m^2h	96.5	65.5	37.5	24.0
50% initial flux, L/m^2h	48.3	32.7	18.8	12.0
Time to 50% initial flux, h	2.6	3.6	3.4	5.7
Time to flux zero, h	5.4	7.6	7.2	12.4
Total amount of permeate until flux 50% of J_0, kg	45.0	41.8	22.6	24.1
24h work cycle				
MF (h)	2.6	3.6	3.4	5.7
Cleaning (h)	3.5	3.5	3.5	3.5
Cycle (MF + Cleaning) (h)	6.1	7.1	6.9	9.2
No cycles/24h	4.0	3.4	3.5	2.6
Total h of running/24h	10.1	12.1	11.8	14.9
Total h of cleaning/24h	13.9	11.9	12.2	9.1
Running vs. cleaning time ratio	**0.7**	**1.0**	1.0	1.6
Total amount collected in 24h, kg	**178.5**	**142.2**	78.7	62.7

A physicochemical analysis of the juice obtained under the highest velocity conditions is shown in Table 7. Clear apple juice was obtained after MF under both cross-flow velocities, using a membrane pore size of 0.8 µm. The MF process caused the changes in turbidity, and color, while pH, °Brix and TA (%) were not affected (Table 7). The observed changes were caused by the removal of SIS by the membrane.

Table 7. Physicochemical properties of raw apple cider and MF juice*.

Cross-flow velocity	Product	pH	°Brix	TA (%)	Turbidity (NTU)	SIS (g/L)	Color		
							L	a	b
5.5 m/s	Raw cider	3.70	12.75	0.505±0.006	461±4.2	10.67±1.36	32.15±0.08	5.86±0.02	9.08±0.08
	MF juice	3.70	12.67	0.506±0.005	40±0.1	0.00±0.00	45.34±0.28	1.36±0.09	24.75±0.24
6.5 m/s	Raw cider	3.72	12.68	0.500±0.001	505±1.4	19.98±1.05	31.95±0.11	5.81±0.11	8.87±0.06
	MF juice	3.72	12.68	0.504±0.008	48±0.6	0.00±0.00	45.19±0.08	1.64±0.04	24.98±0.09

*Values are only available for cross-flow velocities of 5.5 m/s and 6.3 m/s (one MF replicate).

Conclusions

MF processed was optimized in this study and MF at a cross-flow velocity of 5.5 m/s and TMP of 159 kPa was found to have high flux and low flux decline over time. Taking into consideration of cleaning cycle in a 24 hour projection, energy and utility consumption, it was recommended to use this condition as the optimal condition for the MF system in this study.

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4.2 THE EFFECT OF APPLE CIDER CHARACTERISTICS AND MEMBRANE PORE SIZE ON MEMBRANE FOULING

Abstract

In this study, the effect of membrane pore size on the permeate flux data and physicochemical properties of apple juice obtained by microfiltration (MF) was investigated. Four different membrane pore sizes were used: 0.2, 0.45, 0.8 and 1.4 μm. MF using membrane pore sizes of 0.2 and 0.45 μm resulted in similar flux behavior and physicochemical properties of the MF juice, while MF using membrane pore sizes of 0.8 and 1.4 μm resulted in similar juice properties and permeate flux. Clear juices were obtained using all four pore sizes, and no suspended insoluble solids were detected after MF. MF did not induce any changes in pH and °Brix, for any of the four pore sizes. Pectin retention in the MF juice decreased with increasing membrane pore size. Based on these results, it was hypothesized that different fouling mechanisms occur during MF, depending on the membrane pore size. For MF using 0.2 and 0.45 μm pore size, surface fouling and cake layer formation were the dominant fouling mechanisms, while for MF using 0.2 and 0.45 μm pore constriction and pore blocking were dominant. Fouling was attributed to the haze particles in cider, and their size relative to the pore size of the membrane. This information can be used to optimize MF processes able to deliver apple juice of desired properties, and maximize the permeate flux in MF of apple cider.

Introduction

Microfiltration (MF) has received increased interest in recent years as a method for juice clarification and microbial removal. Compared to traditional processing methods, MF can maintain high nutritional and sensory quality of the processed juice. One of the challenges

associated with the large scale adoption of MF by the juice industry is the significant decline in permeate flux with time due to membrane fouling. In general, membrane fouling mechanisms include pore constriction, pore blocking and cake layer formation (de Barros, Andrade, Mendes, & Peres, 2003). Particles in the feed that are smaller than the membrane pore openings can enter the pores; if they get adsorbed onto the membrane channels they will cause pore constriction, thus reducing the effective diameter of the pores. When feed particles are comparable in size with the membrane pore size, pore blocking occurs as the permeate flux creates a convective drag toward the membrane, and particles are adsorbed and/or deposited onto the membrane pores and surface. Particles larger in size than the membrane pores can be retained onto the membrane surface as a cake layer. A significant reduction of the permeate flux will occur in all of these cases. The specific mechanisms of membrane fouling depend on the feed composition and the interactions between the membrane material and the feed components. Several studies examined the effect of small pore MF (0.1 to 0.2 µm membrane pore size) on the permeate flux and the composition of MF apple juice (Fukumoto, Delaquis, & Girard, 1998; Padilla-Zakour & McLellan, 1993; Su, Liu, & Wiley, 1993; Vladisavljević, Vukosavljević, & Bukvić, 2003; Wu, Zall, & Tzeng, 1990; Ben Amar, Gupta, & Jaffrin, 1990; Zárate-Rodríguez, Ortega-Rivas, & Barbosa-Cánovas, 2001). Wu, Zall, & Tzeng, (1990) showed that MF membranes with a 0.1 µm pore size had a higher permeate flux than UF membranes with molecular weight cut-off of 5 kDa and 50 kDa, and the MF juice had significantly higher total soluble solids, was visually darker and was preferred over UF juice by a sensory panel. This suggests that an even larger pore size can lead to a further increase in flux and transmission of nutritional components a membrane pore size larger than 0.2 µm, which is typically used by the juice industry for clarification

purposes, but the permeate flux and the composition of the MF product in large pore MF of apple cider cannot be predicted based on what is known for small pore MF.

Therefore, the objective of this study is to evaluate the effect of apple cider components and membrane pore size on the flux and fouling in large pore MF of apple cider.

Materials and Methods

Materials

Cold, raw apple cider was obtained from Cornell Orchards (Ithaca, NY) and Red Jacket Orchards (Geneva, NY). Cider was stored at 4 °C for maximum of two weeks before being processed. Due to the seasonal production, some batches of pasteurized cider were stored frozen at -20 °C and thawed at 4 °C before use.

Microfiltration experiments

The pilot-scale microfiltration unit consisted of a 50 gallon feed tank connected to a variable-speed centrifugal pump, a tubular heat exchanger and a ISOFLUX™ tubular ceramic membrane of TAMI design (GEA Filtration, WI) placed inside a stainless steel housing. The membrane had an outside diameter of 25 mm, a length of 1200 mm, 23 internal channel of 3.5 mm hydraulic diameter each, and a total membrane surface area of 0.35 m^2. Four membrane pore sizes were used in this study: 0.2 μm, 0.45 μm, 0.8 μm, and 1.4μm.

The MF of raw apple cider was conducted at a cross-flow velocity of 5.5 m/s and a temperature of 6±1 °C, which was maintained by circulating chilled water in the counter current tubular heat exchanger. The temperature was chosen to preserve the quality of apple cider and MF juice and limit browning during processing.

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

$$TMP = \frac{(P_1 + P_2)}{2} - P_p \qquad (1)$$

All pressures were expressed in kPa. The permeate pressure (P_p) equaled the atmospheric pressure. The transmembrane pressure in all MF runs was 159 kPa.

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

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

where: *J*: permeate flux (L/m^{2}h); *M*: amount of permeate (kg) collected in the time interval *t* (h); A: surface area of the membrane (m^2); ρ: density of the permeate at the filtration temperature (kg/m^3).

The duration of the MF experiments was 1 h.

In order to compare the rate of flux drop among different experimental conditions, the relative flux was calculated as:

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

where: *J*: permeate flux at a given time point (L/m^{2}h); J_o: initial flux (L/m^{2}h).

The "initial flux value", J_0, was taken at 7 min after starting the pump, after the system was fully stabilized. The value of the relative flux relates to membrane fouling, i.e. a lower J/J_0 value indicates more pronounced fouling of the membrane.

Membrane cleaning

After each MF experiment, 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 HNO_3 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 membrane before and after the MF experiments.

Physicochemical analysis of cider and juice

pH was measured at 20 °C using a Fisher Scientific Accumet Excel XL20 pH meter, (Fisher Scientific, Pittsburgh, PA). **°Brix** was measured with a MISCO® digital probe refractometer (MISCO® Products Division, Cleveland, OH) at room temperature.

Viscosity was measured at 6 °C using a Brookfield DV-II+ Pro viscometer with a ULV adapter, in triplicate.

Turbidity of the apple cider was measured before and after the MF process using a 2020wi turbidimeter (LaMotte Company, USA) in Formazin Nephelometric Units (FNU). Measurements were duplicated.

The **suspended insoluble solids (SIS)** content was determined by taking 10 mL of apple cider sample and centrifuging at 2200 x g for 15 min; after discarding the supernatant, the sediment (SIS) was weighed and SIS was expressed in g/L. All experiments were carried out in duplicate.

The **pectin** content was determined with a colorimetric assay using m-hydroxydiphenyl for analysis of galacturonic acid (Kintner & van Buren, 1982), in duplicate.

The **particle size distribution** in the apple cider and MF juice was measured 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) and size distribution was converted from the experimental data. 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 to be in the range of hundreds of nm. No dilutions were made to raw apple cider samples and the microfiltered juice samples. Each particle size measurement is consisted of 8 individual runs for duration of 30 s per run. The relative particle size distribution and the intensity weighted effective diameter were determined for each sample. At least one measurement for each sample was conducted and measurements were taken within 24 h. It is important to note that this method of particle size measurement works on the assumption that all particles have spherical shapes.

The **zeta potential** of apple cider and juice was also measured, using the Zeta Potential attachment of the Brookhaven 90Plus Particle Size Analyzer.

Zeta potential of the ceramic membrane used in this study was measured using a Malvern Zetasizer nano-ZS (Malvern Instruments Ltd., Malvern, UK) with disposable folded capillary cells (Malvern Instruments Ltd., Malvern, UK). Two grams of ceramic membrane was ground into a fine powder using a pestle and mortar. The powder was then suspended into 10 mL deionized water (Mili-Q; Merck Millipore Ltd., Billerica, MA), vortexed thoroughly, and allowed to settle for 40 min without disturbance. Next, 0.1 mL of the supernatant was transferred

into 0.9 mL of pH 3.5 buffer to make a 1:10 dilution. A 1 mL sample from the resulting suspension was aliquoted into a cuvette, and zeta potential were measured at 20 °C, in triplicate, using 100 cycles per analysis for each of the triplicates.

Scanning electron microscopy (SEM) Control membrane surface (0.8 µm) and a fouled membrane surface (0.8 µm) by deposited raw apple cider were examined by SEM. The apple cider components and control membrane surface were fixated onto the membrane surface by treatment with 2.5% (w/v) glutaraldehyde in 0.05 M sodium cacodylate buffer for 1 h. Samples were rinsed in cacodylate buffer three times, 5 min each, and subjected to a secondary fixation step using 1% (w/v) osmium tetroxide in cacodylate buffer for 0.5 h. Samples were rinsed in cacodylate buffer 3 times as described above, and then dehydrated using gradient ethanol solutions of 25% (v/v), 50%, 70%, 95%, 100% and 100% for 10 min each. Samples in 100% ethanol were critical point-dried with carbon dioxide. Dried surfaces were mounted to SEM stubs with carbon tape and coated with evaporated carbon. A Zeiss LEO 1550 field emission SEM was used. A voltage of 1-5 kV was used depending on the specific sample. Images were acquired using the accompanying software SmartSEM (Carl Zeiss Microscopy, LLC, Germany).

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.

Results and Discussion

Effect of membrane pore size on MF flux

A decline of **permeate flux** over time occurred for all membrane pore sizes. An example of flux graph is illustrated in Figure 5. For all four pore sizes, a change in relative flux occurred over the duration of the MF run, which indicated the occurrence of membrane fouling. As seen in Figure 6, the behavior was different for the two smaller pore size membranes (0.2 and 0.45 µm), as compared to the two larger pore size membranes (0.8 µm and 1.4 µm). The initial flux for MF of ciders ranged from 39 L/m^2h to 58 L/m^2h, and 39 L/m^2h to 83 L/m^2h when apple cider was MF with membrane pore size of 0.2 and 0.45 µm, and from about 60 L/m^2h to 90 L/m^2h for the 0.8 µm and 1.4 µm membranes. The final flux (flux at 1 h) varied between 38 - 44 L/m^2h and between 33 - 54 L/m^2h for 0.2 and 0.45 µm pore sizes, and from approximately 40 L/m^2h to 70 L/m^2h , for the 0.8 and 1.4 µm pore sizes, significantly higher than that of 0.2 and 0.45 µm pore sizes. MF using 0.2 and 0.45 µm pore sizes resulted however in a higher relative flux after 1 h of MF (86% and 82% of the initial flux) than for 0.8 µm and 1.4 µm membranes (75% of the initial flux).

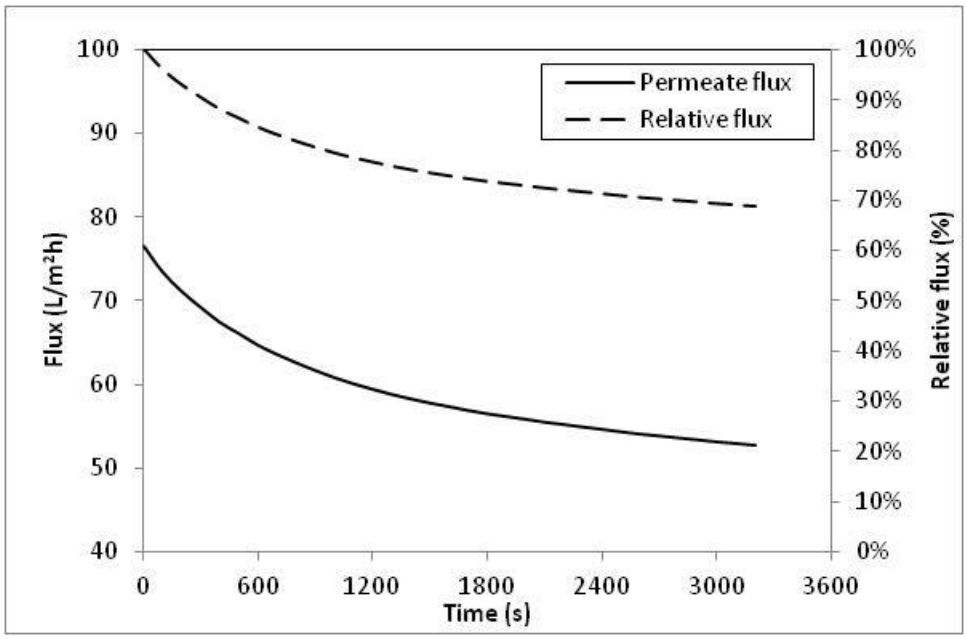

Figure 5. Evolution of permeate flux and relative flux with time. Data for MF using 0.45 µm pore size membrane and a cider turbidity of 677 FUN.

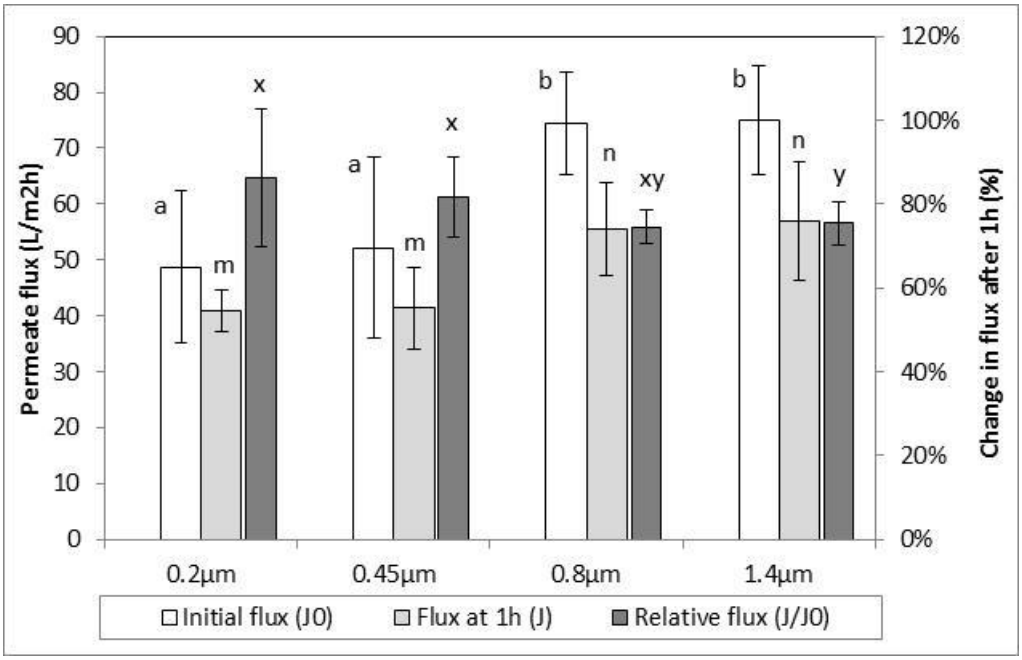

Figure 6. Initial flux, flux at 1 h, and relative flux after 1h of MF with ceramic membranes with pore sizes of 0.2 µm, 0.45 µm, 0.8 µm and 1.4 µm. Within a data series, data points not connected by the same letter are statistically different from each other ($P<0.05$).

To summarize, the 0.8 µm and 1.4 µm pore size membranes had the highest initial and final (1h) fluxes, due to a greater mass transfer through the large size pores, but also manifested a higher propensity for fouling as compared to the 0.2 µm and 0.45 µm pore size membranes. For the duration of the MF runs conducted in this study the slower fouling rate for the 0.2 and 0.45 µm membrane was not sufficient to compensate the higher flux obtained using the 0.8 µm and 1.4 µm. Overall, this data indicates that pore size is critical for overall MF performance when evaluating both flux and relative flux change. The fact that flux did not increase linearly with membrane pore size suggests different fouling mechanisms for the different membrane pore sizes.

Effect of membrane pore size on juice quality

MF of apple cider with all membrane pore sizes resulted in a bright, visually clear apple juice. No significant differences between the pH and °Brix of the cider and MF juice were found, for any pore size (**Table 8**). SIS was completely removed by MF, for all membrane pore sizes. MF juice had a lower viscosity than apple cider for all membrane pore sizes, although the difference was statistically significant only for the 0.45 µm pore size. The change in viscosity was clearly affected by the pore size of the MF membrane, with the reduction in viscosity being directly correlated with the pore size, ranging from 38.78% for 0.2 µm to 4.21% for 1.4 µm. This can be attributed to the removal of colloidal particles in apple cider. As mentioned by Genovese & Lozano (2006), the viscosity of cloudy apple juice (apple cider) is given by the characteristics of the serum and dispersed colloidal particles, and the interaction between them. The repulsive electrostatic and hydration interaction forces between the dispersed colloidal particles contribute significantly to the viscosity of cloudy apple juice (Genovese & Lozano, 2006), and thus their removal by MF is expected to result in a decrease in viscosity.

Table 8. Physicochemical characteristics of unprocessed apple cider and apple juice microfiltered using different pore size membranes. Values within the same column not connected by the same coefficient are statistically different from each other (P<0.05).

Property \ Pore size	Product	0.2µm	0.45µm	0.8µm	1.4µm
pH	Raw cider	3.73±0.10	3.69±0.21	3.74±0.01	3.70±0.14
	MF juice	3.76±0.04	3.72±0.18	3.73±0.19	3.71±0.14
°Brix	Raw cider	12.13±1.24	12.96±0.69	12.78±0.15	12.64±0.51
	MF juice	12.00±0.82	13.20±0.78	13.11±0.22	13.03±0.51
Viscosity ($\times 10^{-3}$Pa·s)	Raw cider	5.08±0.54	3.09±0.72 (a)	3.33±0.60	3.09±0.69
	MF juice	3.11±0.94	2.35±0.28 (b)	3.08±0.61	2.96±0.52
	Change	*-38.78%*	*-23.95%*	*-7.51%*	*-4.21%*
Turbidity	Raw cider	1035±342	922±292	920.65±257.45	926.50±297.09
	MF juice	1.37±0.31 (c)	1.55±0.64 (c)	55.10±18.68 (d)	54.03±11.43 (d)
SIS (g/L)	Raw cider	13.86±0.89	18.50±3.44	19.34±2.03	19.04±4.29
	MF juice	None	None	None	None
Pectin content (mg/100g)	Raw cider	92.65±9.43	109.96±109.30	83.67±31.73	89.75±36.37
	MF juice	5.57±3.56	21.91±31.96	75.18±34.79	70.96±36.56
Pectin transmission (%)	MF juice	6.23±4.47 (e)	20.90±14.00 (e)	86.12±22.21 (f)	83.76±21.15 (f)

The removal of particles from cider by MF also resulted in a decrease in turbidity, observed both visually and instrumentally. The turbidity of raw apple cider ranged from 677 FNU to 1669 FNU. MF juice had a significantly lower turbidity when using 0.2 µm (1.37 FNU

average) and 0.45 µm pore sizes (1.55 FNU average) than for the 0.8 µm and 1.4 µm pore sizes (~55 FNU average) (**Table 8**).

The pectin content of the raw apple cider used in this study varied widely, from 26.85 mg/100 g to 390.24 mg/100 g. A large variation in pectin content among different cloudy apple juices is considered normal, and is due to different cultivars and stage of maturity of the apples (Markowski, J.,Baron, A.,Mieszczakowska, M.,Plocharski, W., 2009). A significantly lower pectin content was found in the permeate juice from MF with 0.2 and 0.45 µm pore sizes (3 to 9%, and 6 to 51% of the initial pectin content, respectively) as compared to the 0.8 µm pore size (45-100% of the initial pectin content) and the 1.4 µm pore size (60 to 100% of the initial pectin content) (**Table 8**). This suggests that the majority of pectic materials were able to pass through the larger pore size membranes (0.8 and 1.4µm), but not through the 0.2 and 0.45µm membranes. For the latter, the majority of the pectin from apple cider was rejected.

Effect of apple cider particles on fouling

Membrane fouling occurred for all membrane pore sizes, as indicated by the drop of flux with time (Figure 5). SEM images of ceramic membrane used in MF of apple cider suggest that this was caused both by components of raw cider deposited on the membrane surface (external fouling), as well as some cider components adsorbed into the structure of the ceramic membrane material (internal fouling) (Figure 7). In Figure 7(a), the regularly shaped rods represent ceramic particles that form the membrane porous structure. The large, spherical particles shown in Figure 7(b) represent starch granules. Starch granules in apple cider are generally not considered to contribute to membrane fouling (Riedl, Girard, & Lencki, 1998), since their size (4.1 µm to 12 µm diameter) is much larger than the membrane pore sizes (Singh, Inouchi, & Nishinari, 2005).

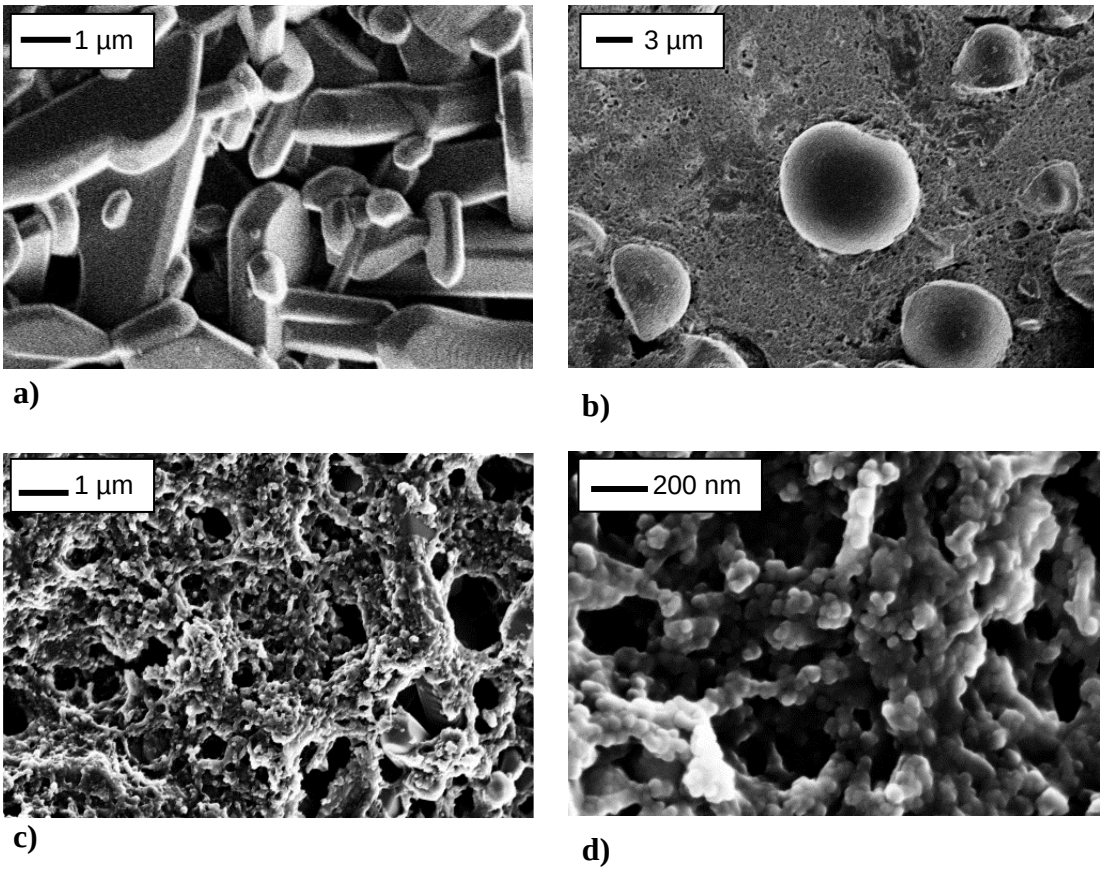

Figure 7. SEM image of a 0.8µm ceramic membrane: (a) clean membrane surface; (b) - (d) apple cider components deposited onto the membrane surface, at different magnifications. Spherical particles in (b) represent starch granules.

The effective diameters of haze particles in apple cider used in this study ranged between 800-2300 nm, with an average effective diameter of particles of 1083 nm ± 333 nm, which was consistent with previous findings by (Genovese & Lozano, 2000) (50 -3000 nm). Two populations of particles were observed: one group with sizes larger than 1000 nm (Group 1 particles) and the other group with sizes below 1000 nm (Group 2 particles). An example of particle size distribution in apple cider is shown in Figure 8. Microorganisms inherently

occurring in raw apple cider may also be accounted in the particle size measurement and contributed to the particle size distribution and fouling in -this study.

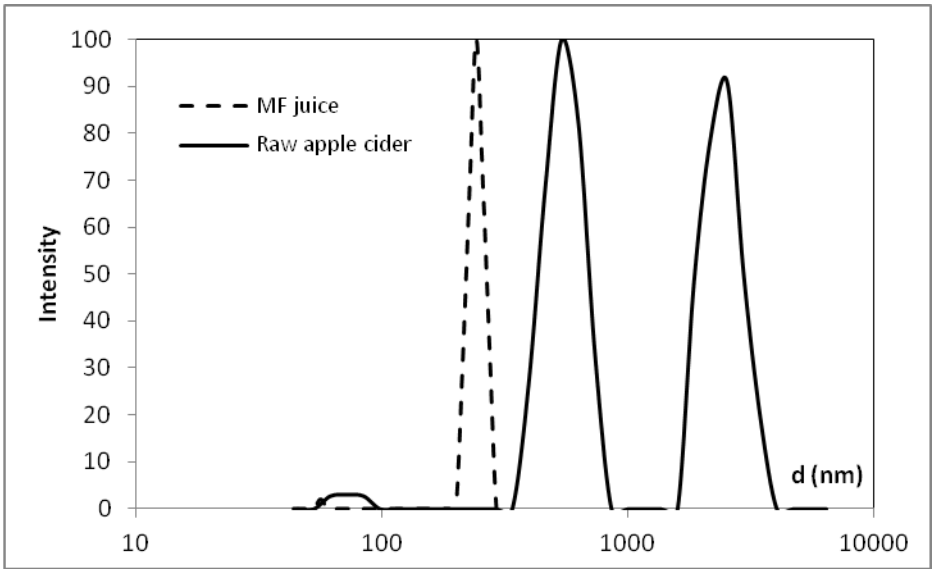

Figure 8. Example of particle size distribution in raw apple cider and MF juice obtained from MF with a 0.45 µm pore size membrane

After MF, apple juice filtered with the 0.8 µm and 1.4 µm membranes had particles with similar effective average diameters (614 and 641 nm, respectively), which represent 77% and 46% of the nominal pore size of the membranes, respectively. MF using 0.45 µm resulted in an average effective diameter for the MF juice of 345 nm, which represents 77% of the nominal pore size of the membrane. Juice obtained from MF using 0.2 µm membrane had an average effective diameter of 371 nm, which is larger than the membrane pore size and thus may be due to the aggregation of small haze particles. There is a statistically significant difference between particle sizes of MF juice using large pore sizes (0.8 and 1.4 µm) and small pore sizes (0.2 and 0.45 µm) (Figure 9). This suggests that fouling by haze particles from apple cider significantly reduced the effective pore size of the MF membranes, which resulted in haze particles larger than the effective membrane pore sizes being rejected by the membrane.

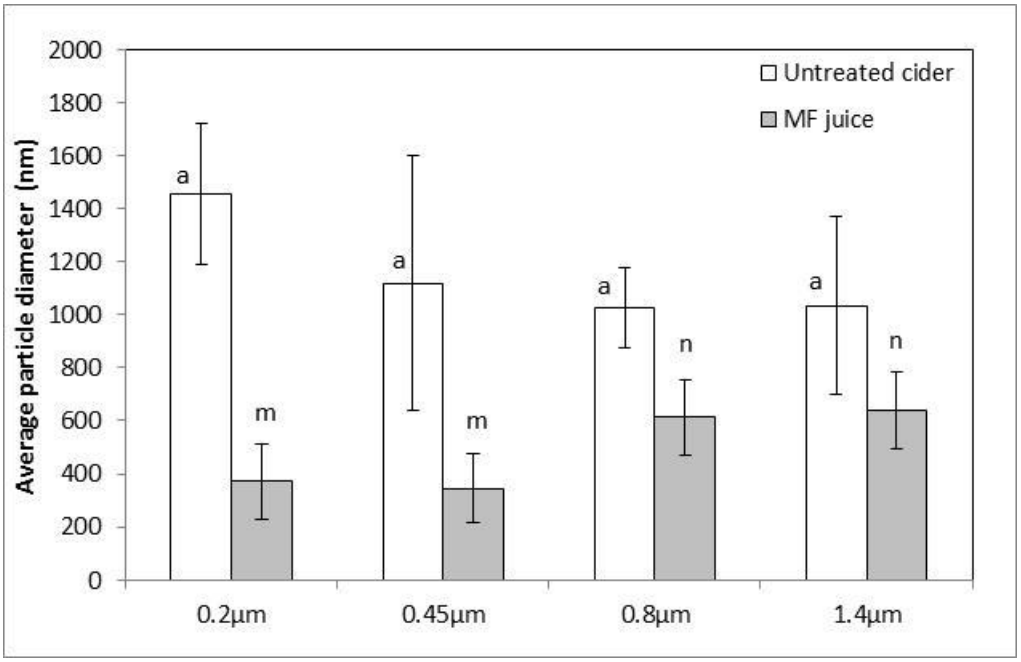

Figure 9. Particle sizes (effective average diameters) measured in raw apple cider and MF juice. Within a data series, data points not connected by the same letter are statistically different from each other ($P<0.05$).

When relating to the flux data using four membrane pore sizes, it can be inferred that Group 1 haze particles caused primarily internal pore adsorption and/or partial pore blockage for the 0.8 µm and 1.4 µm membranes, since their size is comparable to the pore size of these membranes. Meanwhile, the fouling mechanism for 0.2 and 0.45 µm membrane may be dominated by cake layer formation on the membrane surface. This also explains the almost complete rejection of pectin by the smaller size membranes.

Therefore, it is proposed here that when the colloidal particle size (haze particles) in apple cider approaches the pore size of the membrane the fouling mechanism transitions from the dominance of pore blockage and cake formation (smaller pore size membranes) to internal pore constriction and partial pore blockage (for the larger pore size membranes). The identification of

this critical pore size can help membrane selection depending on the properties and particle size distribution of a particular feed material or cider cultivar blend, which will greatly improve the performance of MF in industrial applications.

Besides the effect of particle size of apple cider on membrane fouling, it is also important to consider the affinity of the particles in apple cider for the ceramic membrane. Zeta (ξ) potential was used as a measure of the electrical charge of the colloidal particles in cider, and of the electrical charge of the membrane material. The ξ-potential values of haze particles measured for all apple cider and juice samples varied, but were all negative (varied in a large range from -0.17 to -9.59 mV), which is consistent with the findings reported by (Benitez & Lozano, 2006)). The ξ-potential of the ceramic materials at a typical apple cider pH measured -8.53 ± 1.18 mV. There was a slight electrical repulsion between haze particles and the membrane material, but it was not a strong one to prevent particle adsorption onto the membrane material and effectively prevent fouling.

Conclusions

MF is an efficient and effective process to clarify apple cider and other juices. Different fouling mechanisms of MF using different membrane pore sizes were illustrated, thus a selection of membrane pore sizes should be considered during MF process of apple cider and juices and its optimization. Further shelf-life and sensory studies should be conducted to investigate the qualities of apple juice microfiltered by large MF membrane pore sizes to find the optimized process condition that offers the desirable organoleptic quality of juices.

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4.3 ROLE OF PECTIN AND HAZE PARTICLES IN MEMBRANE FOULING DURING APPLE CIDER MICROFILTRATION

Abstract

Membrane fouling limits the commercial use of microfiltration (MF) as a method for clarification and microbial removal from apple cider. In this study, the specific role of pectin in membrane fouling during MF of apple cider was investigated. Apple juice with four different concentrations of pectin was obtained and subjected to cold MF with pore sizes above 0.45 μm. The physicochemical properties of the juice before and after MF and the permeate flux were quantified. The experimental data demonstrated that pectin plays a significant role in fouling during MF of apple cider, and its fouling effect increased with increasing concentration. Most significant for fouling is the association of pectin with other components in apple cider, which results in haze particles with low surface electrical charge, which seem to be the main culprit for membrane fouling during MF of apple cider. The effect of pectin hydrolysis prior to MF of apple cider on membrane fouling during MF was also evaluated. Depectinization was beneficial to MF using membrane pore sizes below 0.45 μm, for which the fouling mechanisms is dominated by cake layer formation. Yet, depectinization had a negative effect on MF of apple cider 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. The findings of this study have practical implications for the development of efficient, commercially attractive MF processes for apple cider.

Introduction

Microfiltration (MF) is a pressure-driven process that can clarify liquid streams by physically removing suspended solids that are larger than the membrane pore size, also known as a "sieving effect". MF is used in the juice industry primarily as a clarification method, as an alternative to the conventional juice fining and filtration methods such as filtration using sheets and diatomaceous earth. The benefits of using this technology include its continuous operation, no need for filter aids, and the ability of the membranes to be chemically cleaned and regenerated. In addition to clarification, MF can remove many of the microorganisms of concern from the feed, including bacteria, yeast, mold and protozoa (Zhao et al., 2015).

A significant issue that limits the commercial use of membrane filtration in general and MF in particular, 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 in size than the membrane pores, they can be retained in a cake layer formed onto the membrane surface.

In MF of apple cider, haze particles and pectin are believed to have a significant role in membrane fouling. Colloidal haze particles in apple cider are formed by 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). 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).

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 size also retain low molecular weight components such as pectin and chemical compounds that contribute to juice color and flavor, thus stripping the final product of some of its most desirable properties (Wu, Zall, & Tzeng, 1990). Therefore, using membrane larger pore sizes could offer the advantage of an increased retention of the juice nutritional, color and flavor components, as well as a potentially higher permeate flux than 0.2 μm membranes. Nonetheless, the fouling mechanisms and specifically the role of pectin in fouling during large pore MF have not yet been elucidated. A good and clear understanding of these mechanisms can provide an insight into ways to mitigate fouling and make MF of apple cider a commercially attractive process. The main goal of this study is to develop a better understanding of the role of haze particles and pectin in membrane fouling during large pore MF.

Materials and Methods

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.

Pectin addition experiments

In order 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 microfiltration membrane, to remove most of the suspended solids. Apple pectin with MW 30,000-100,000 (Sigma Aldrich, St. Louis, MO) was then added to this clarified juice in a range of concentration typically found in apple cider, at the target concentrations of 0.05%, 0.10%, 0.15% and 0.20% w/w (denoted as levels I to IV).

Pectinase treatments

The effect of pectin hydrolysis on fouling and permeate flux during MF of apple cider 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.

Microfiltration processing

A pilot-scale MF unit consisting of a 50 gallon 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 m^2. The membrane pore sizes

(the nominal pore diameter according to the membrane manufacturer) used in this study were 0.2 µm (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 was calculated as:

$$TMP = \frac{(P_1 + P_2)}{2} - P_p \qquad (1)$$

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:

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

where: *J*: permeate flux (L/m^{2}h); *M*: amount of permeate (L) 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.

The relative flux was calculated as follows:

$$Relative\ flux = \frac{J}{J_o} \times 100\% \qquad (3)$$

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

The value of the relative flux relates to membrane fouling, i.e. a lower J/J_0 indicates a more pronounced fouling of the membrane than a higher J/J_0 value. This normalized parameter allows direct comparisons among MF experiments that have different permeate flux values.

The MF process was conducted at a cross-flow velocity of 5.5 m/s and a transmembrane pressure (TMP) of 159 kPa, which were selected based on the conclusions of a previous study (Zhao and Moraru, 2015). 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., 2015).

Membrane cleaning

After each MF experiment, 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 HNO_3 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.

Physicochemical analysis

The physicochemical 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) and size distribution was converted from the experimental data. 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 to be 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 method particle size measurement 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 30s per run. At least one measurement for each sample was conducted, and measurements were taken within 24 h from processing.

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

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.

Results and Discussion

Effect of pectin concentration on MF and fouling

3.1.1. Effect of pectin addition on juice properties

In a previous study that investigated the mechanisms of membrane fouling in MF of apple cider it has been shown that fouling mechanisms are similar for the 0.2 µm & 0.45 µm pore sizes on one hand and 0.8 µm & 1.4 µm on the other hand (Zhao and Moraru, 2015). 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 the lower pore size and 0.8 µm for the larger pore size. These pore sizes were also chosen based on their

potential to significantly reduce microbial pathogenic and spoilage organisms in apple cider (Zhao et al., 2015).

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 approach 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 concentration typically found in apple cider. The actual pectin concentrations are shown in Table 9. The addition of pectin to the base juice did not induce any changes in the pH and °Brix of the juice (Table 9), as expected. It is important to note that most of the added pectin passed through the MF membranes. As seen in Figure 10, the 0.8 µm pore size allowed for a higher level of transmission than the 0.45 µm membrane. A linear regression analyses 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.

Table 9. Physicochemical properties of juice with the addition of different concentrations of pectin, before and after MF. Values represent averages of two runs (with 3 analytical replicates per run) ± 1 stdev.

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

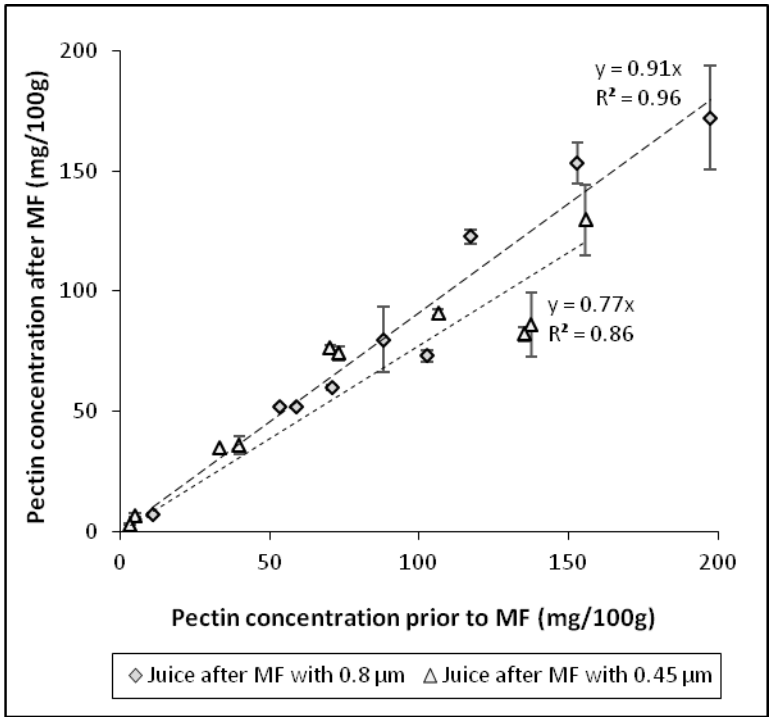

Figure 10. Pectin transmission through the membrane after MF with 0.45µm and 0.8 µm pore sizes. Data points correspond to two processing runs per pore size. Individual data points represent averages of three analytical replicates ±1 stdev.

An evaluation of the particle size of a pectin dispersion in distilled water indicated the presence of two distinct populations of particles (Figure 11, upper): a class of particles with an average diameter smaller than 500 nm, and a class of micrometer sized particles, presumably pectin aggregates. 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, 2015). It should be noted that the particle size data in Figure 11 is based on the signal intensity, and although some conclusions can be drawn from this data regarding the proportion of particles in each size class, the peaks cannot be related quantitatively to the amount of pectin in each size class. Two classes of particles were also found in the juice with added pectin. While the small particles were found in

the same size range regardless of the level of pectin addition, the larger particles increased in size as the pectin concentration/ 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). 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 extensively reported (Lopes da Silva and Rao, 2006).

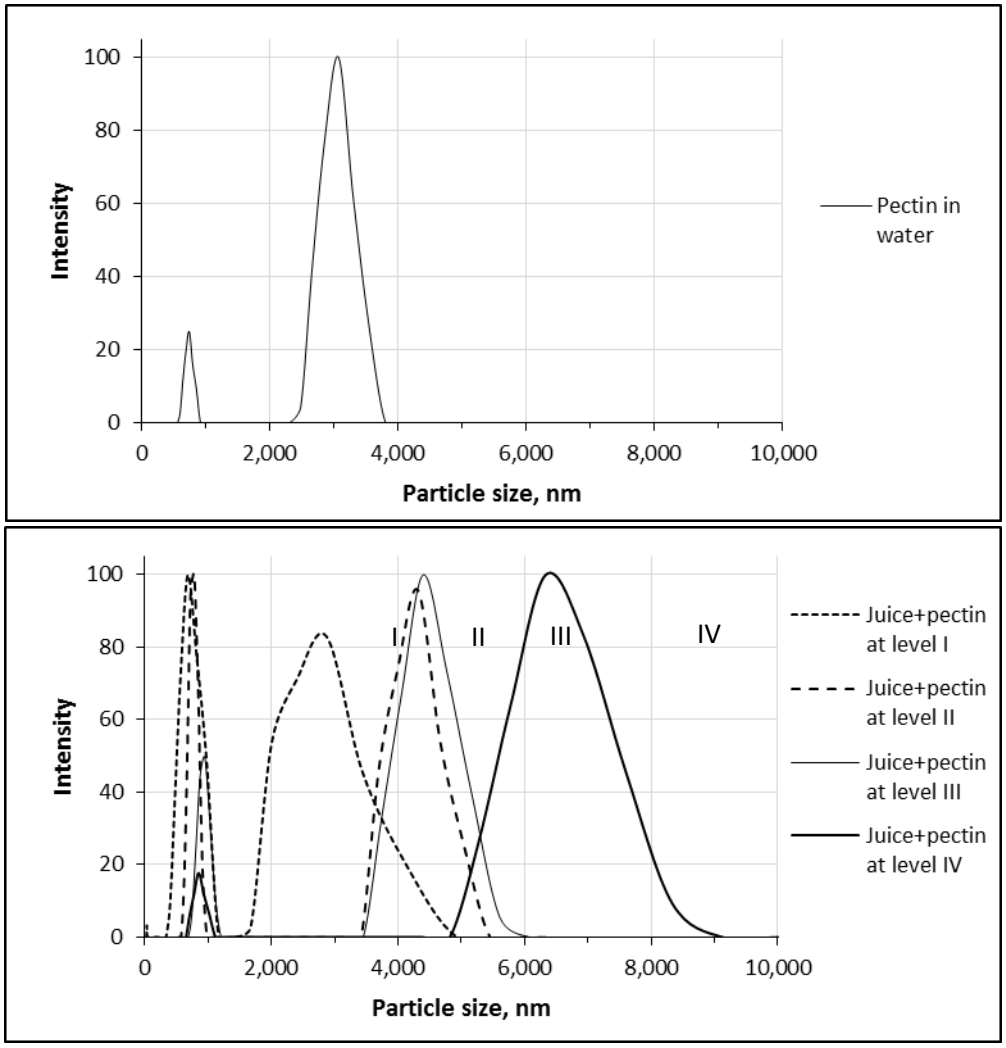

Figure 11. Examples of particle size distribution for a dispersion of pectin in distilled water (upper) and for base juice with added pectin at levels I through IV (lower), prior to MF.

The increase in aggregation with pectin concentration was also reflected in the quantitative particle size analyses. As seen in Figure 12, the average particle diameter in the juice with added pectin (prior to MF) increased linearly with concentration until about 100 mg pectin / 100 g, and reached a plateau above that. As a note, the trend lines in Figure 12 are used 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 MF 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 576.5 nm. 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/ 100g, and then reached a plateau, at a particle size of about 1000 nm. The limit particle size found for both cases of MF 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, either involving pectin-pectin interactions or pectin-polyphenol interactions.

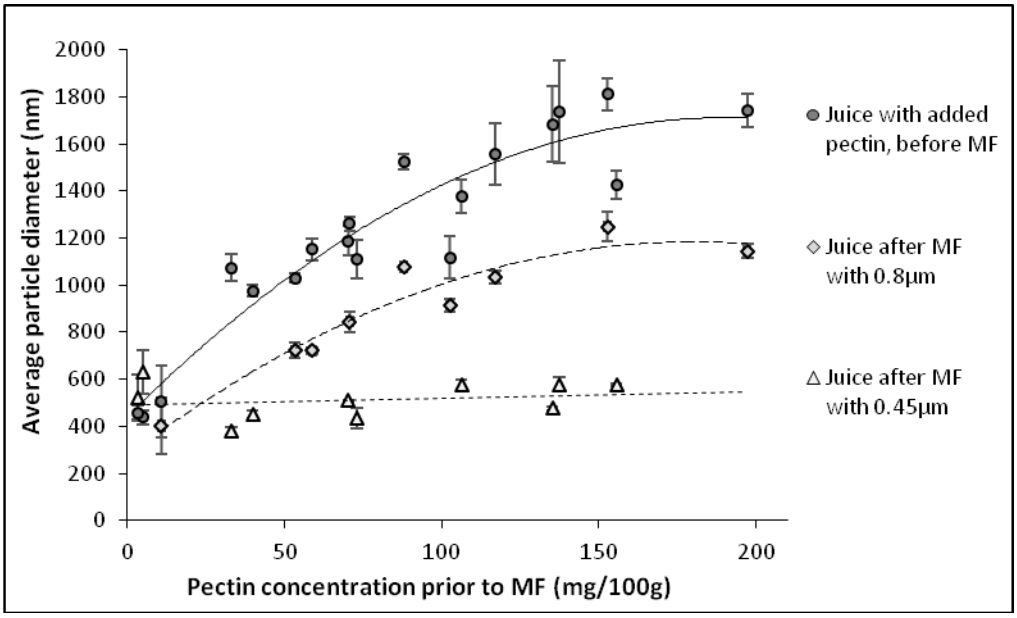

Figure 12. Relationship between particle size and pectin concentration in juice before and after MF. Data points correspond to two processing runs per pore size. Individual data points represent averages of three analytical replicates ±1 stdev.

The pectin concentration and particle size had a direct effect on some physicochemical properties of the 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 (Figure 13). 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.

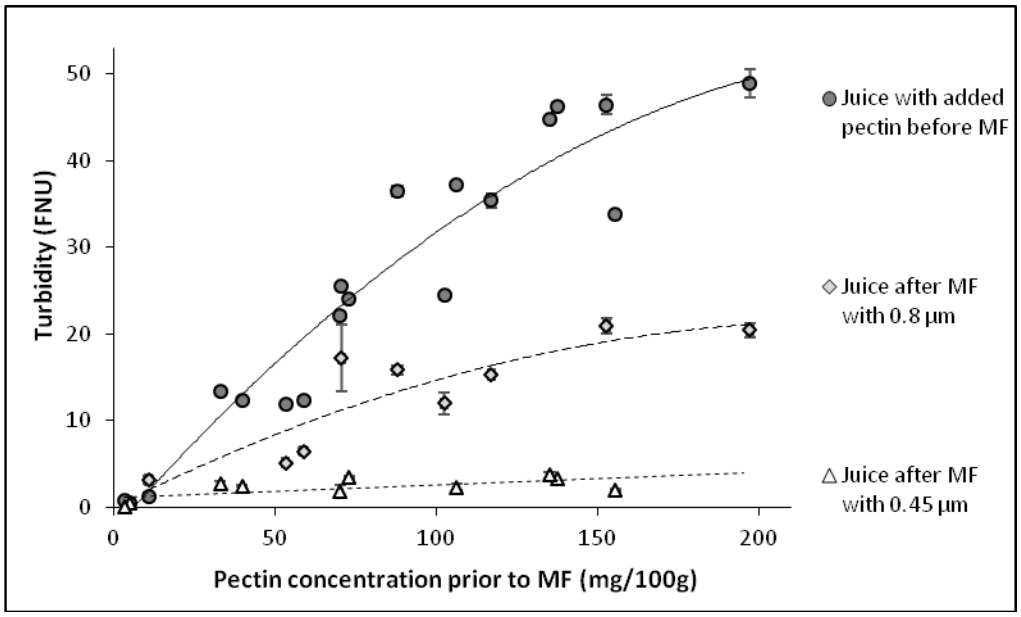

Figure 13. Relationship between turbidity and pectin concentration in juice before and after MF. Data points correspond to two processing runs per pore size. Individual data points represent averages of three analytical replicates ±1 stdev.

Pectin concentration also impacted directly the shear viscosity of the juice, with viscosity increasing linearly with pectin concentration (Figure 14). 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 of 7% for MF with 0.8 µm pore size. These values are consistent with those obtained in our previous MF 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, 2015).

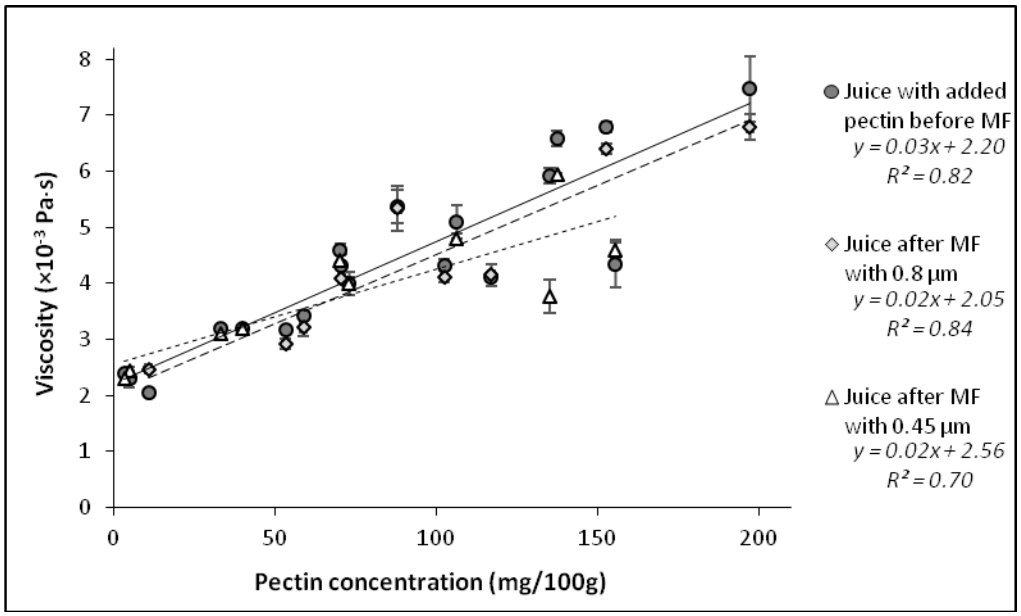

Figure 14. Shear viscosity (at 6°C) of juice before and after MF as a function of pectin concentration. Data points correspond to two processing runs per pore size. Individual data points represent averages of three analytical replicates ±1 stdev.

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 Figure 15, 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 with 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 (Figure 15). A clear decrease in the magnitude of ζ was observed after MF, particularly for the 0.45 µm pore size (Figure 15, lower). 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.

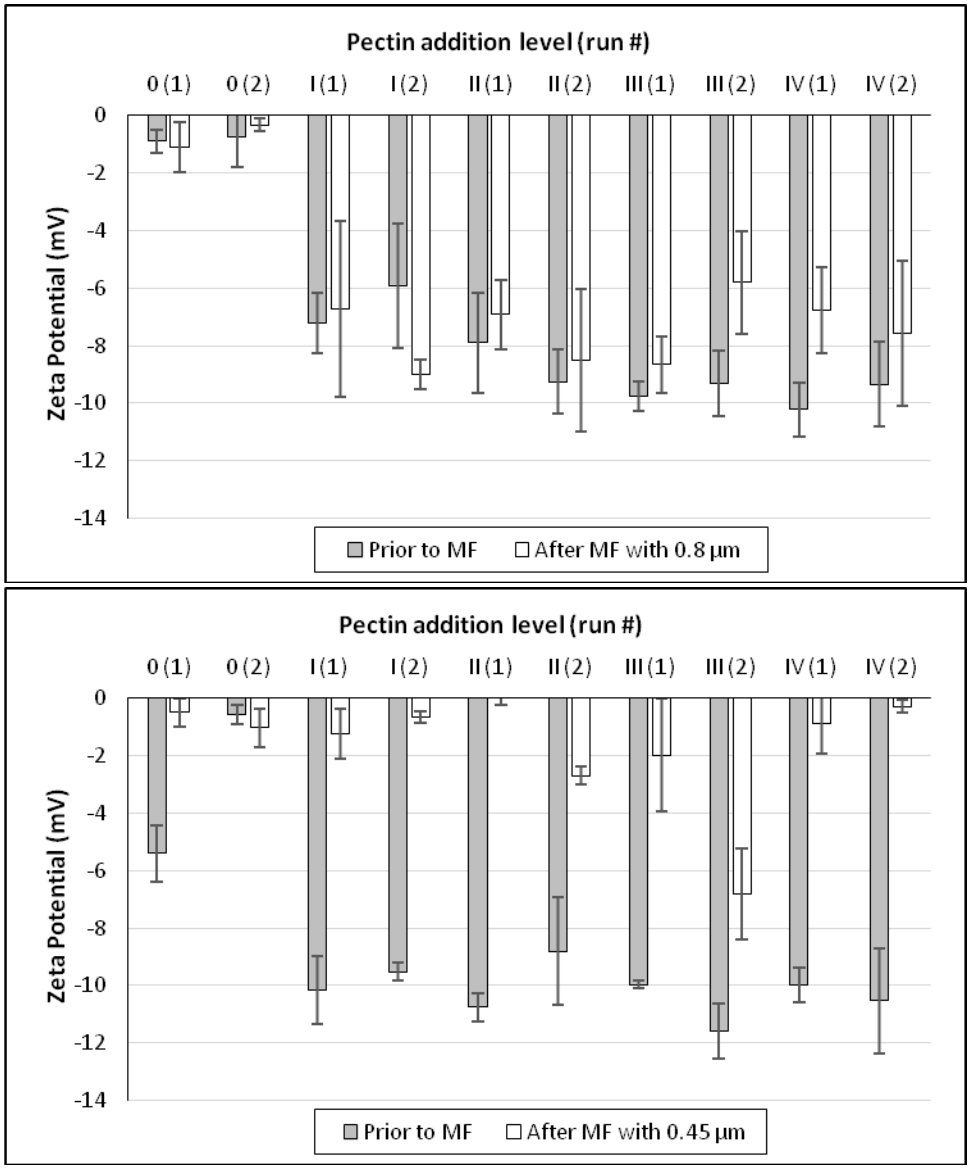

Figure 15. Zeta potential measured in juice before and after MF with 0.8 µm (upper) and 0.45 µm (lower), at different levels of pectin addition (I through IV). Data points correspond to two processing runs per pore size (1 and 2). Individual data points represent averages of three analytical replicates ± 1 stdev.

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 permeate flux during MF. Permeate flux decreased with the increase of pectin concentration in the feed for both pore sizes

(Figure 16). Similar results were reported from a previous study using an apple flavored model system 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. Figure 16 shows that an inverse exponential relationship was found between feed viscosity and permeate flux, 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 evaluated. This demonstrates a clear influence of pectin on membrane fouling during MF of apple juice / cider.

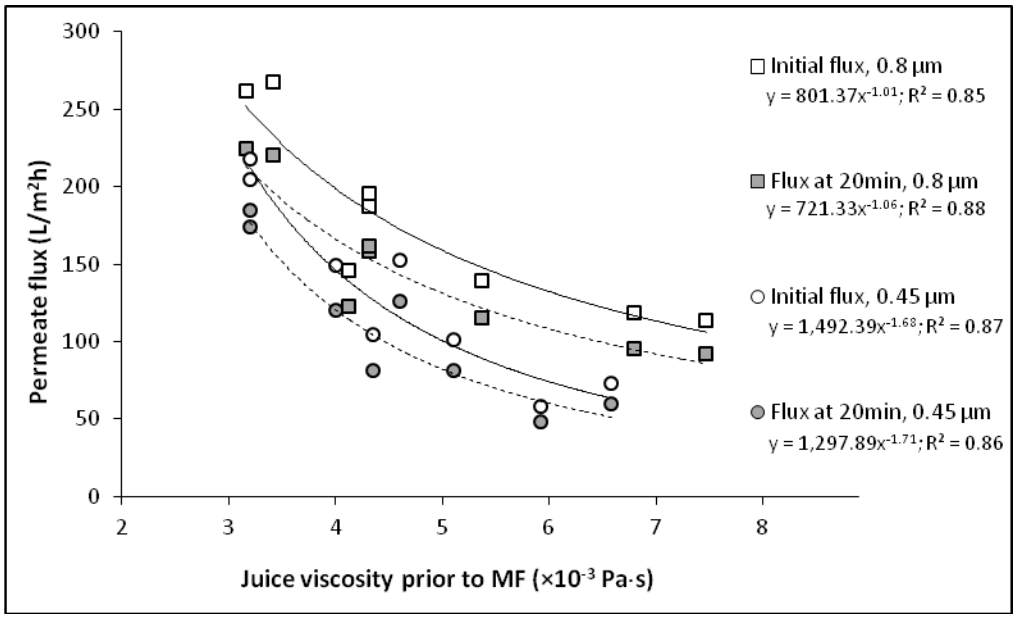

Figure 16. Relationship between permeate flux and pectin concentration in the juice prior to MF. Data points correspond to two processing runs per pectin addition level and pore size.

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 (Figure 17), 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 membrane pore sizes. This indicates that besides the actual pectin content, the other juice components, particularly polyphenols and proteins, and their interactions with pectin, have even a 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 explains the very low charge, indicated by the low magnitude of ζ, measured both in the juice without added pectin (Figure 15) and in the untreated apple cider (Table 10). The ζ of the ceramic membrane, measured in a previous study (Zhao et al., 2015) 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 (externally or internally) 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.

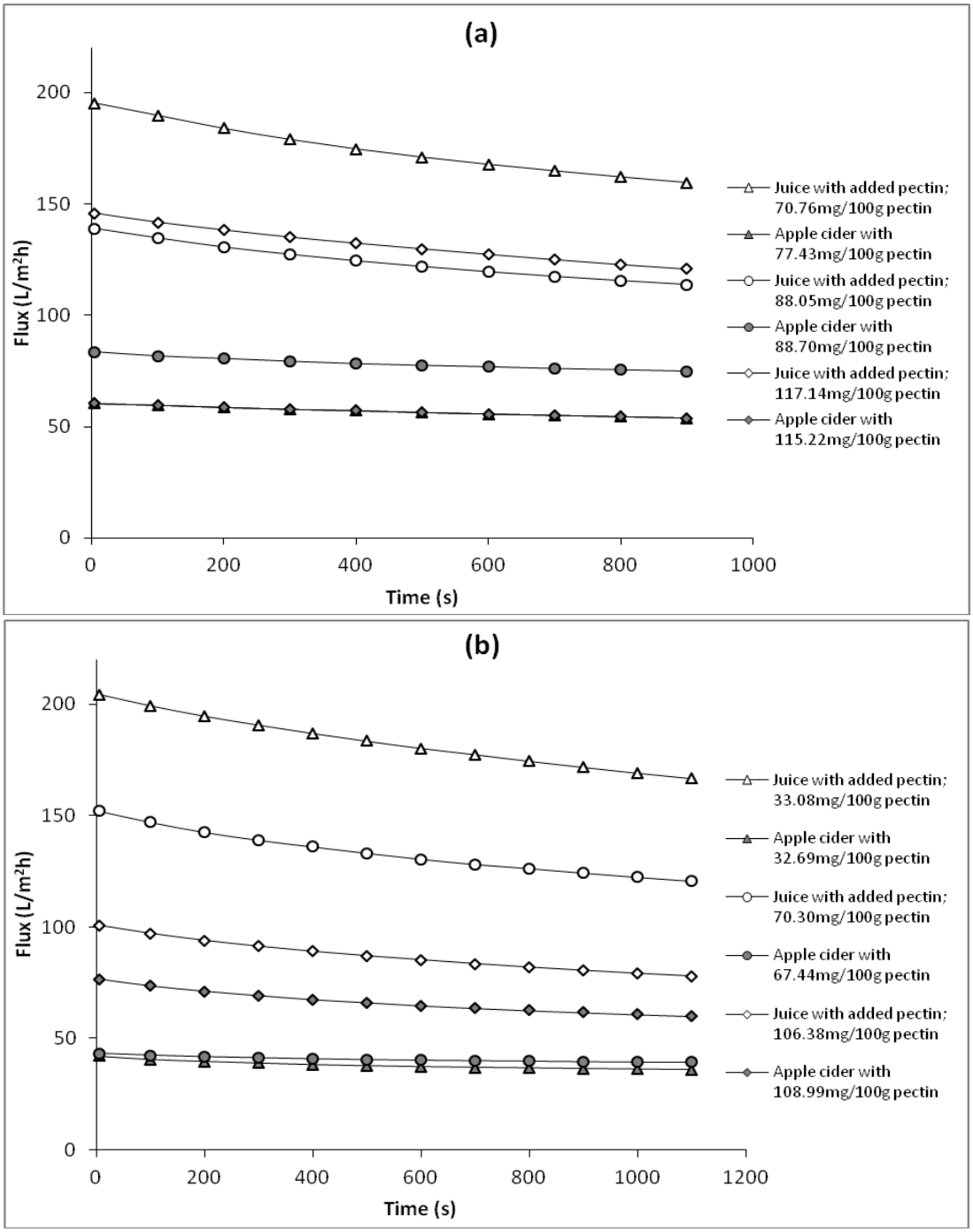

Figure 17. Flux curves for MF of apple cider with natural composition and MF of apple juice with added pectin, at similar pectin concentration, using (a) 0.8 µm membrane pore size and (b) 0.45 µm membrane pore size.

Table 10. Physicochemical properties of apple cider, before and after depectinization with various enzymes. Values represent averages of three analytical replicates ± 1 sdtev

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

Effect of depectinization on MF and fouling

Previous studies have shown that depectinization can improve the performance of apple cider membrane filtration (Su, Liu, & Wiley, 1993; Wu, Zall, & Tzeng, 1990; Padilla-Zakour & McLellan, 1993). However, 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 is shown in Table 10. Polygalacturonase (PG) was chosen for subsequent treatments, since it showed the largest reduction in particle size.

It was previously reported that during depectinization the enzyme attacks the pectin coat of the haze particles, which exposing the positively charged protein core and allowing electrostatic aggregation of haze particles (Yamasaki et al., 1967; Beveridge, 1997). Although no changes in zeta potential were observed after the enzymatic treatment in this study (Table 11), 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 transformations that took place 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 11), which suggests that some of the pectic materials may have precipitated out of solution as a result of 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 11).

Table 11. Physicochemical properties of apple cider (before MF) and apple juice (after MF) in the depectinization study. Values represent averages of two runs (with 3 analytical replicates per run) ± 1 sdtev.

Property	Treatment	0.45 µm MF pore size		0.8 µm MF pore size		1.4 µm MF pore size	
		Untreated	**PG treated**	**Untreated**	**PG treated**	**Untreated**	**PG treated**
Pectin content (mg/100g)	**Before MF**	109.96±109.30	44.89±27.65	83.67±31.73	38.21±14.21	89.75±36.37	40.30±0.70
	After MF	21.91±31.96 *(21% transmission)*	35.66±21.27 *(80% transmission)*	75.18±34.79 *(86% transmission)*	28.49±5.76 *(77% transmission)*	70.96±36.56 *(84% transmission)*	38.22±5.90 *(95% transmission)*
pH	**Before MF**	3.69±0.21	3.73±0.01	3.74±0.01	3.78±0.00	3.70±0.14	3.76±0.01
	After MF	3.72±0.18	3.74±0.01	3.73±0.19	3.81±0.06	3.71±0.14	3.77±0.03
Soluble solids (°Brix)	**Before MF**	12.96±0.69	14.00±0.00	12.78±0.15	13.83±0.24	12.64±0.51	13.75±0.35
	After MF	13.20±0.78	14.25±0.35	13.11±0.22	13.63±1.24	13.03±0.51	14.25±0.35
SIS (g/L)	**Before MF**	18.50±3.44	22.37±3.65	19.34±2.03	27.04±2.91	19.04±4.29	25.30±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±0.72	2.65±0.08	3.33±0.60	2.54±0.16	3.09±0.69	2.58±0.14
	After MF	2.35±0.28	2.47±0.08	3.08±0.61	2.44±0.19	2.96±0.52	2.44±0.12
Turbidity (FNU)	**Before MF**	922±292	1661±45	920±257	2024±612	927±297	1617±74
	After MF	1.65±0.64	0.58±0.01	55.10±18.68	4.68±1.06	54.03±11.43	11.12±0.36
Particle size (nm)	**Before MF**	1120±479	958±3	1027±614	1318±160	1035±334	1137±99
	After MF	345±132	0.58±0.01	151±143	173±235	641±1	374±1

MF of depectinized apple cider resulted in visually clear apple juice, and a complete removal of SIS (Table 11). Yet, some differences in the instrumentally measured turbidity were found, with MF juice obtained from PG treated cider having a lower turbidity compared to the juice obtained from untreated cider, especially for the 0.8 µm and 1.4 µm pore sizes (Figure 18). This correlates well with the fact that the MF juice from PG treated cider had significantly smaller particle sizes as compared to the MF juice from untreated cider using the 0.8 µm and 1.4 µm pore sizes (Figure 19).

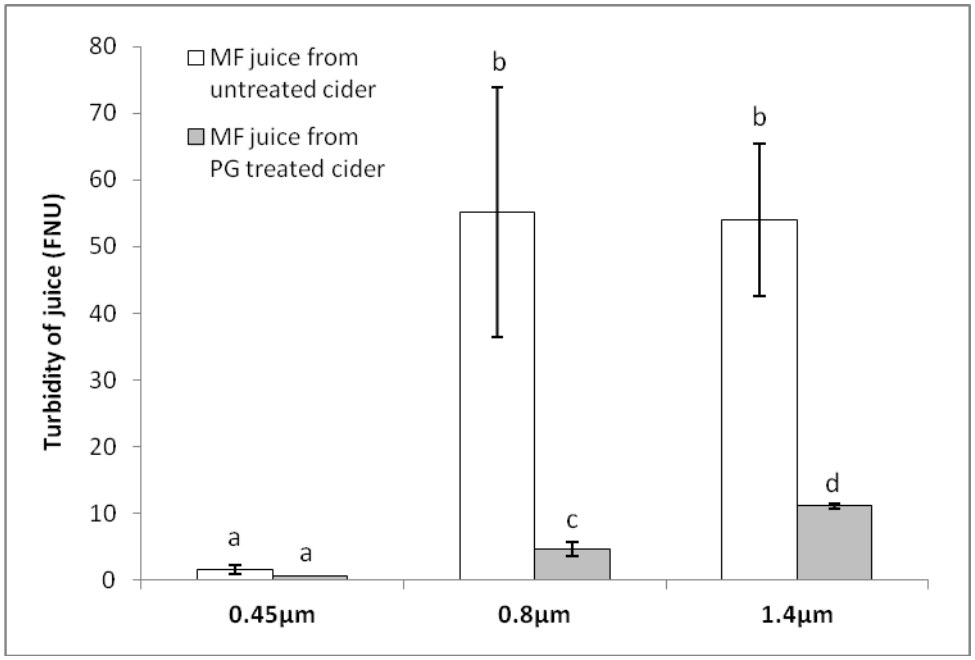

Figure 18. Turbidity of MF juice obtained from untreated and PG treated apple cider. Data points not connected by the same letter are statistically different from each other ($P<0.05$)

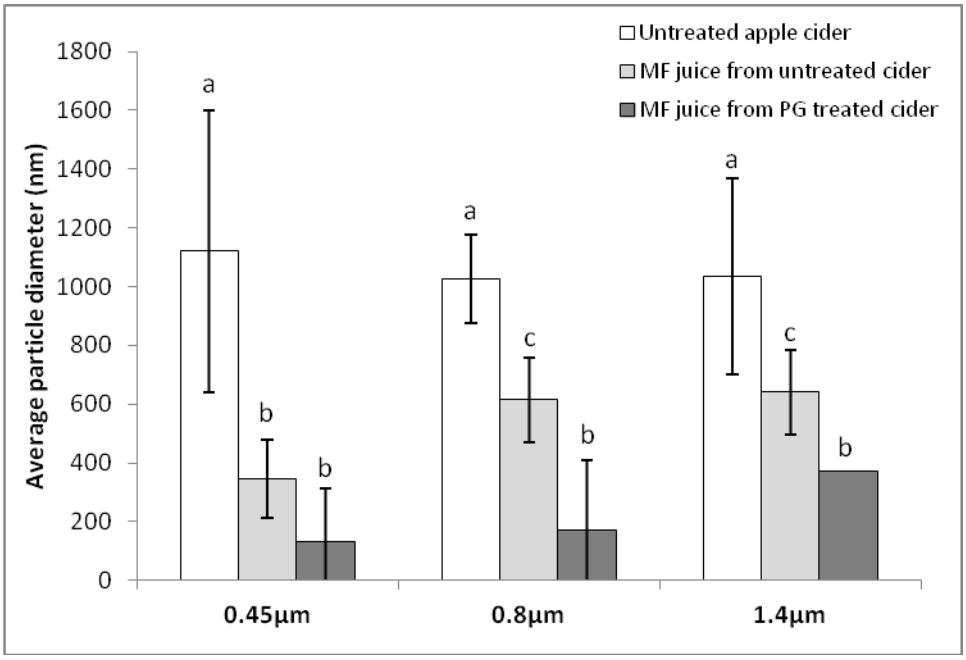

Figure 19. Average particle diameters in untreated apple cider, MF juice from untreated cider and MF juice from PG treated cider. Data points not connected by the same letter are statistically different from each other ($P<0.05$)

PG treatment also caused a significant decrease of cider 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 cider 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 (both initial and after 1h) for the 0.45 µm pore size membrane Figure 20), 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 that using untreated cider (Figure 20). At the same time the fouling rate, expressed by the relative drop in flux, was accelerated by the PG treatment for both 0.45 and 1.4 μm (Figure 21).

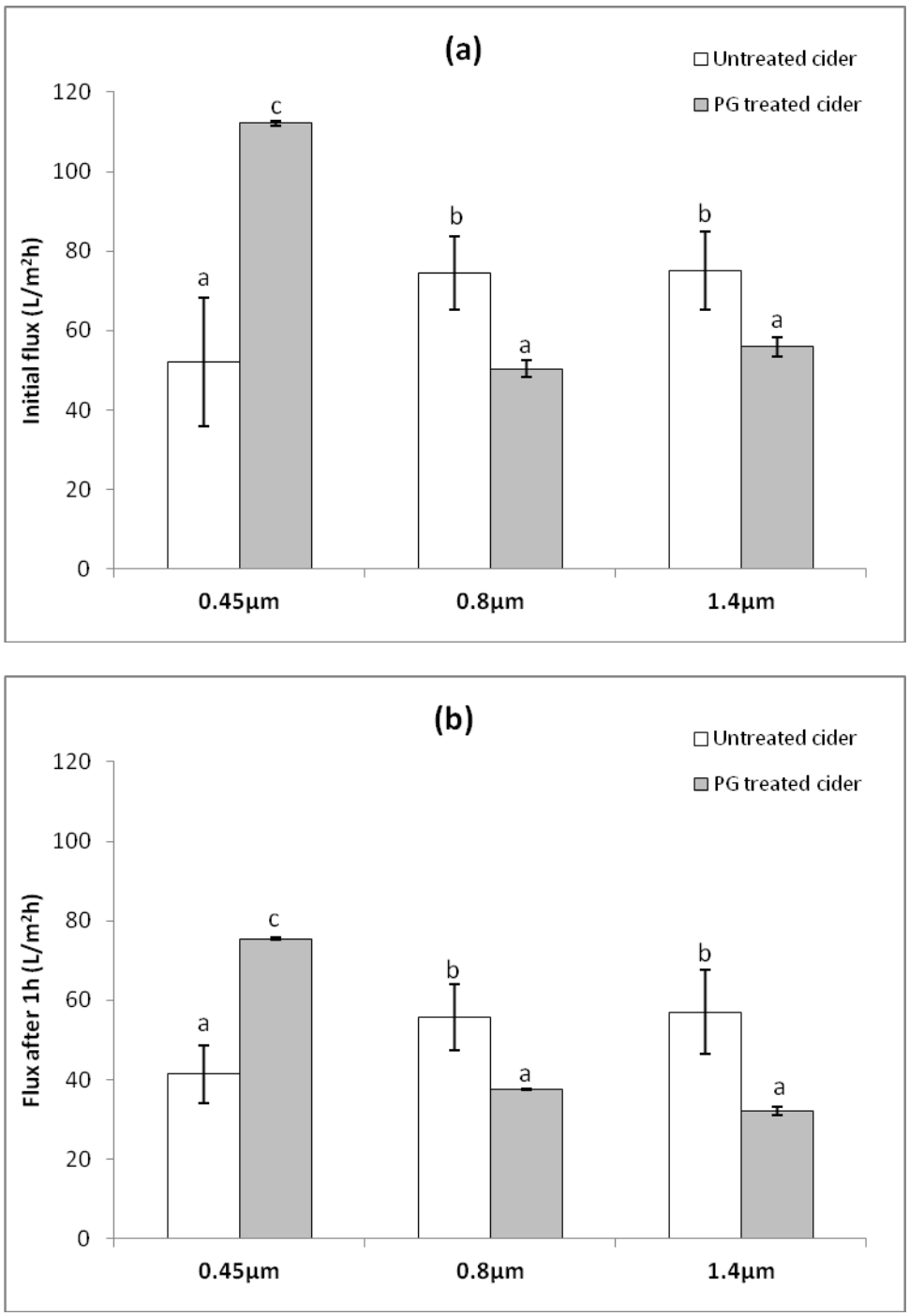

Figure 20. Initial flux (a) and flux after 1h (b) for MF of untreated and PG treated apple cider.

Data points not connected by the same letter are statistically different from each other ($P<0.05$)

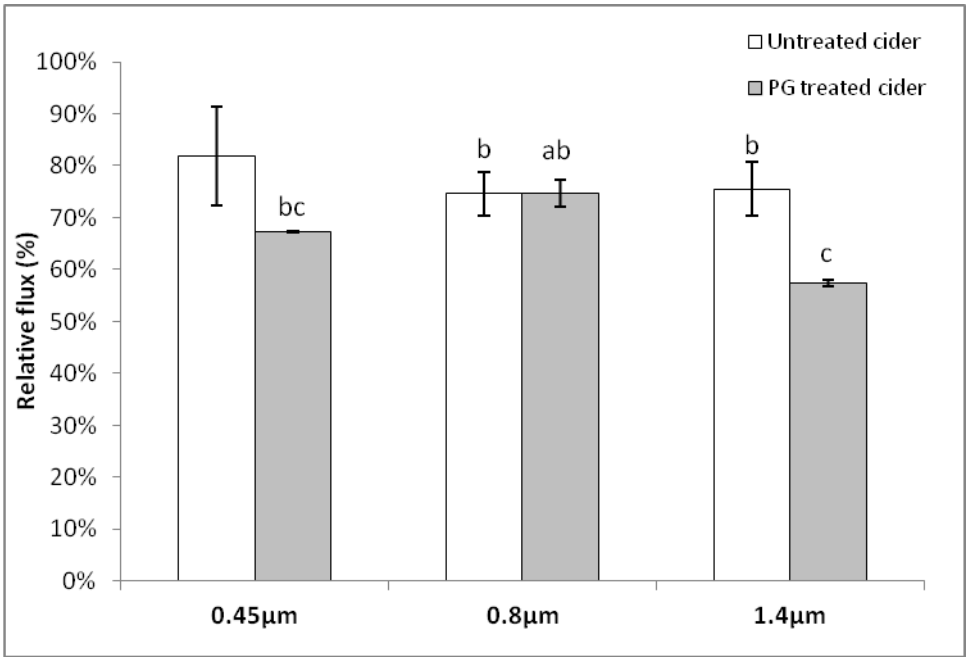

Figure 21. Relative flux after 1h for MF of untreated and PG treated apple cider. Data points not connected by the same letter are statistically different from each other ($P<0.05$).

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 (Zhao and Moraru, 2015) 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. 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 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 showed larger, more open pore structure deposits on membranes as compared to untreated cider.

The observations made in this study are very significant from a practical point of view, since depectinization does not appear to be beneficial for large pore MF (at pore sizes above 0.8 µm), even if it leads to improved fluxes in smaller pore size applications (0.45 µm and lower).

Conclusions

Pectin does play a significant role in fouling during MF of apple cider, and its fouling effect is increasing with increased concentration. Most significantly though it is the association of pectin with other components in apple cider, particularly proteins and polyphenols, which lead to the formation of haze particles with low surface electrical charge, which seem to be the main culprit for membrane fouling during MF of apple cider. Pectinase treatment results in a size reduction and structural changes of haze particle sizes, which appeared beneficial to MF using a membrane pore size below 0.45 µm, for which the fouling mechanisms is dominated by cake layer formation on the membrane surface. However, depectinization did not have a positive effect on MF flux 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 MF should only be used for small pore size MF (<0.45 µm), where this treatment can help reduce fouling and achieve a commercially attractive process performance.

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4.4 Optimization of a CO_2 backpulsing technique for improving the flux in cold MF of apple cider

Abstract

In order to mitigate membrane and improve flux during cold MF of apple cider, a CO_2 backpulsing was tested and the CO_2 backpulsing conditions optimized.

The most significant flux enhancement by CO_2 backpulsing using a 0.2 µm membrane pore size was achieved at a duration of 2 s and a frequency of 2 min. After MF, clear apple juice was obtained. A decrease in viscosity, turbidity, particle size and pectin content of the product due to the complete removal of suspended insoluble solids was also found out.

Introduction

Besides optimizing the process parameters mentioned in Chapter 4.1, other means to mitigate membrane fouling in membrane filtration of juices currently used in industry include pre-treatment of the feed (pectinase treatment), prefiltration, use of uniform TMP, use of turbulence promoters, backwashing, and backpulsing. Efforts are also made by membrane manufacturers to modify the membrane material or treat the membrane surface in order to diminish the interactions with the feed components and thus make it less prone to fouling. Recently, a CO_2 backpulsing technique has been developed and was proven to be an economical way to improve the permeate flux during cold MF of skim milk (Fritsch & Moraru, 2008). A slightly different backpulsing method using air and N_2 was also proven to increase permeate flux in UF of apple juice (Su, Liu, & Wiley, 1993). However, using air can promote oxidation, which may have adverse effects on the color and flavor of the processed apple cider. Using CO_2

backpulsing has a potential to improve filtration flux while preserving juice quality, but the backpulsing conditions (a combination of duration and frequency) may affect the effectiveness of this technique. Therefore, the objective of this study is to optimize CO_2 backpulsing condition as a method to improve flux in cold MF of apple cider.

Materials and Methods

Cold, raw apple cider was obtained from Cornell Orchards (Ithaca, NY), and was stored at 4°C for a maximum two weeks before being processed.

Microfiltration of apple cider. A pilot-scale MF unit consisting of a 50 gallon 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 area of 0.35 m^2. A pore size of 0.2 µm membrane was used.

The MF of raw apple cider was conducted at a temperature of 6 $\pm$ 1°C, by circulating chilled water in a counter current tubular heat exchanger. This temperature was chosen to preserve the quality of apple cider and juice and reduce browning during processing. MF process condition was at 5.5 m/s velocity and TMP of 159 kPa as recommended in Chapter 4.1.

CO_2 backpulsing was conducted at a pressure of 275 kPa through three injection ports along the membrane at time zero for 5.3 hours at duration and interval specified. The conditions of backpulsing duration and interval tested were 1s/2min, 2s/2min, 3s/2min, 1s/3min, 2s/3min and 3s/3min. Control was MF of raw apple cider with no CO_2 injection.

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

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

where: *J*: permeate flux (L/m^{2}h); *M*: amount of permeate (L) 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). Initial flux value was taken at 7 minutes after starting the pump and final flux was taken after one hour of each run.

The relative flux was calculated as follows:

$$Relative\ flux = \frac{J}{J_o} \times 100\% \qquad (3)$$

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

The value of the relative flux relates to membrane fouling, i.e. a lower J/J_0 indicates a more pronounced fouling of the membrane than a higher J/J_0 value. This normalized parameter allows direct comparisons among MF experiments that have different permeate flux values. All MF experiments were duplicated.

Membrane cleaning. After each MF experiment, a chemical cleaning cycle was carried out. The cleaning procedure consisted of a rinse with RO water for 10 minutes, followed by alkaline cleaning with Ultrasil-25 at a concentration of 20g/L at 80 °C for 30 minutes and a second RO water rinse for 10 minutes or until neutrality. Acid cleaning with 5mL/L HNO_3 at 50 °C for 20 minutes was then performed, followed by a third RO water rinse for 10 minutes or until neutrality. The effectiveness of cleaning and change in the membrane performance with time were monitored by determining the water flux before and after the experiments.

The cider and MF juice were subjected to physicochemical analyses, as described below.

pH and soluble solids content (°Brix) of the cider and MF juice were measured using a Fisher Scientific Accumet Excel XL20 pH meter, (Fisher Scientific, Pittsburgh, PA) and a MISCO® digital probe refractometer (MISCO® Products Division, Cleveland, OH) at room temperature

Viscosity was measured at 6°C using a Brookfield DV-II+ Pro viscometer with a ULV adapter. Measurements were in triplicate.

The **suspended insoluble solids (SIS)** content was determined by centrifuging 10 mL of apple cider sample at 2200 x g for 15 min. After discarding the supernatant, the pellet (representing the SIS) was weighed and SIS was calculated and expressed as g/L.

The **pectin** content was determined, in duplicate, with a colorimetric assay using m-hydroxydiphenyl for analysis of galacturonic acid (Kintner & van Buren, 1982).

The **particle size distribution** was measured 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) and size distribution was computed from the experimental data. 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 to be in the range of hundreds of nm. No dilutions were made to juice samples with different pectin concentrations before and after MF. Each particle size measurement is consisted of 8 individual runs for duration of 30s per run. The relative particle size distribution and the intensity weighted effective diameter were determined for each sample.

At least one measurement for each sample was conducted and measurements were taken within 24 hours. It is important to note that both for the particle size measurement and for the discussion of the role of these particles in fouling the assumption that all particles have spherical shapes was used.

Statistical analysis. The experimental 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.

Results and discussion

After MF of apple cider using 0.2 μm membrane with CO_2 backpulsing, clear cider was obtained. Physicochemical properties of the cider and juice were listed in Table 12. Viscosity, turbidity and particle size of juice was significantly reduced after removal of SIS by MF process.

Table 12. Physicochemical properties of cider and juice after MF using 0.2 µm with CO_2 backpulsing.

	pH	°Brix	Viscosity (10-3Pa·s)	SIS (g/L)	Turbidity (FNU)	Particle size (nm)
Raw cider	3.72±0.08	11.91±0.83	4.69±0.74a	16.228±3.156	1047±320c	1503.0±263.4e
MF juice	3.72±0.09	12.04±0.79	2.72±0.35b	None	1.38±0.90d	459.2±125.9f

CO_2 backpulsing was able to achieve flux enhancement at the test conditions of 1s/2min and 2s/2min, but no flux enhancement was observed for backpulsing at 3s/2min and an interval of 3min (Figure 22). The MF flux achieved by CO_2 backpulsing at 2s/2min was the highest among all conditions tested and the rate of flux decline was comparable to the control. Although at backpulsing condition of 1s/2min flux was improved, the rate of flux decline was higher than control. It was unclear why the flux at 3s/2min and frequency of 3min was lower than control, but the rate of flux decline was smaller than control. This indicated that gas backpulsing at 3s/2min and frequency of 3min for 1s, 2s and 3s may have disrupted or delayed the buildup of fouling layer, but did not effectively improve MF performance. Therefore, the optimal CO_2 backpulsing condition was at duration of 2s and a frequency of 2min.

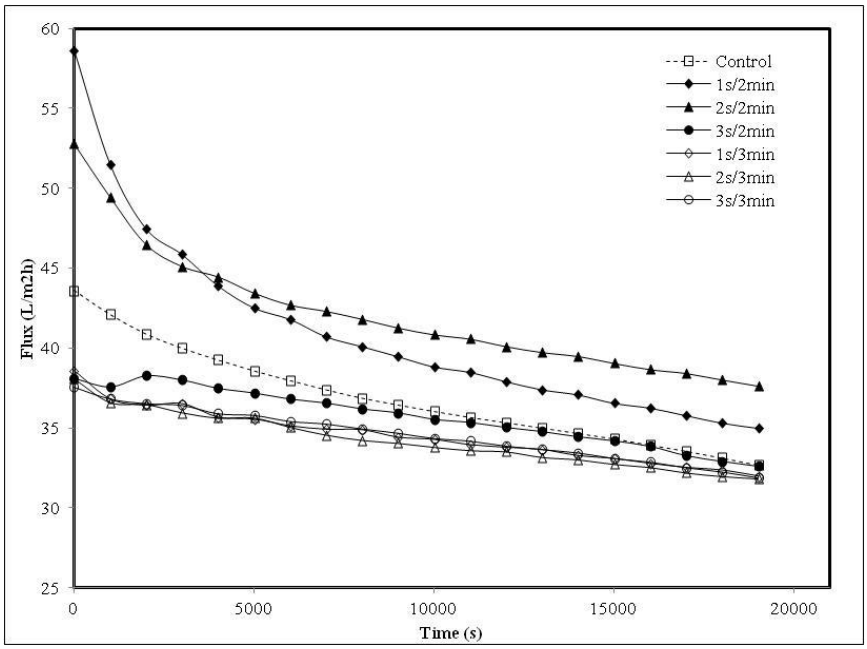

Figure 22. Flux of MF of apple cider using 0.2 µm membrane at different CO_2 backpulsing conditions and control (without CO_2 backpulsing).

Conclusions

Clear apple juice was obtained after MF, and significant changes in turbidity, color, viscosity and particle size were observed because of complete removal of SIS by the membrane. The optimal process parameters for cold MF of apple cider were identified. MF using a ceramic membrane of 0.8 µm at a cross-flow velocity of 5.5 m/s and TMP of 159 kPa at 6 °C resulted in a high flux value and a reduced rate of fouling, and thus were selected as the optimal process conditions. CO_2 backpulsing has the potential to significantly improve MF flux. In this study, the optimal backpulsing duration and frequency was found to be 2s/2min to maintain a high flux and low rate of flux decline. Future studies need to evaluate the effect of CO_2 backpulsing on MF of apple cider, for different membrane pore sizes.

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