M. Kupetz, M. Weber, H. Kollmannsberger, B. Sacher and T. Becker

Impact of Fatty Acids and Medium Chain Fatty Acid Ethyl Esters on the Beer Crossflow Membrane Filtration

Membrane filtration represents a difficult process due to complex beer composition and its interactions with filter materials. Therefore, influences of fatty acids in general and medium chain fatty acid (MCFA) ethyl esters on crossflow membrane filtration have been investigated. During crossflow filtration trials, transmembrane pressure (*TMP*) rise as well as filterability were examined in laboratory scale. In an additional step, beer samples were mixed with MCFA ethyl esters or antifoam agent containing high amounts of fatty acids, resulting in an average decreasing filterability of 20 % as well as a faster pressure rise in crossflow membrane filtration. A significant correlation ($r = 0.99$, $P < 0.05$) between *TMP-rise* and filterability using PES-membranes could be observed. Beer analysis revealed high decrease of β-glucan (up to 150 mg/l) during the first filtration hour. The fluorometric β-glucan method showed a weak correlation to *TMP* increase ($r = –0.77$), whereas colorimetric method exhibited a more distinct connection ($r = –0.93$). Furthermore, the amount of 3-Methylbutyl acetate underwent only slight changes in reference and fatty acid enriched samples, whereas the content in MCFA ethyl ester spiked beer decreased up to 40 %. In addition, content of Ethyl octanoate (30 %) and Ethyl decanoate (40–60 %) dropped during filtration in all samples. The observed results allow specific conclusions regarding filtration performance of beer in crossflow membrane filtration.

Descriptors: β-Glucan; filterability; Esser-test; volatiles; viscosity

1 Introduction

The processing of cereal containing food and beverages set operators to different challenges because of the complex matrices and their rheological behaviour. Particularly in beer production these rheological properties can affect the quality of the final products, mainly noticeable during clarification processes such as lautering and filtration. Beer filtration can be performed using cake (e.g. diatomaceous earth filtration) or surface (e. g. membrane filtration) filtration methods. Both types of filtration are influenced by chemical beer composition, consisting of proteins, polysaccharides, polyphenols, melanoidins and mineral substances as well as microbial cells like yeast [16, 30, 35]. Although similar substance groups are involved in the blockage of filter pores, membrane and diatomaceous earth (DE) filtration are not directly comparable [19]. In particular, membrane filtration is characterized by procedural difficulties due to a rigid membrane separation layer. The applied crossflow-membrane filtration (CFMF) systems are operating with a flow parallel to membrane surface, resulting in the formation of a constant surface layer [1, 5]. This reversible surface layer, mostly consisting of yeast cells, acts as a secondary membrane and retains aggregates from beer [35]. Besides this surface layer resistance, CFMF processes can be characterized by crossflow velocity, transmembrane pressure (*TMP*) as well as membrane resistance. Further influencing factors are size distribution, shape, agglomeration behaviour and surface properties of the suspended particles [26, 35]. Intermittent adsorption and fouling processes can be affected by the selection of membrane material [24]. In addition to different polymer membranes (e. g. polyethersulphone, polyamide), ceramic membranes with nominal pore sizes between 0.2 and 0.65 µm are applied, resulting in a sterile product due to the larger cell dimensions of *Saccharomyces* yeasts and other microorganisms [17, 35].

Because of its pressure and pH resistance as well as the possibility of high flow rates, the membrane material polyethersulphone (PES) has been well-proven in brewing industry [24, 27, 35]. Furthermore, PES has a low affinity to biomacromolecules (e. g. colloids) [35]. Nevertheless, several authors have observed decreasing filter performance because of membrane fouling [10, 28, 30, 32, 36, 39]. In addition to adsorption effects of different protein molecular sizes [12, 18, 33], negative impact of polysaccharides could be shown [7, 14, 32, 36]. The main focus was placed on high molecular weight (HMW) β-glucans as well as other cell wall polysaccharides like arabinoxylans [14, 28, 32]. Correlations between concentration dependent molecular weight (> 90 kDa, $r = –0.62$, $P < 0.001$ [23]) as well as intrinsic viscosity ($R^2 = 0.846$ [21]) of β-glucans on filterability could be examined. Although various substance groups could be

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Abbreviations:

A_m	[m²]	Filter area of the membrane
CFMF		Crossflow-membrane filtration
C_N		Carbon number
CN		Cellulose nitrate
DE		Diatomaceous earth
G	[g]	Filtrate weight
G_{max}	[g]	Maximum filtrate weight
HMW		High molecular weight
$J_{20\ °C}$	[l/m²/bar/h]	Flux
MCFA		Medium chain fatty acid
PA		Polyamide
PES		Polyethersulphone
$P_{filtrate}$	[bar]	Pressure filtrate
P_{inlet}	[bar]	Pressure membrane inlet
P_{outlet}	[bar]	Pressure membrane outlet
Q_P	[l/h]	Permeate flow
RT_N		Retention time of the alkane
RT_{N+1}		Retention time of the next alkane
RT_X		Retention time of the unknown analyte
t	[s]	Filtrate time
T	[°C]	Temperature
TCF	[1/°C]	Temperature correction factor
TMP	[bar]	Trans-membrane pressure

identified affecting membrane filtration performance, the research topic is not finally solved. Despite the compliance of thresholds for e. g. β-glucan or yeast cell count in beer, a spontaneous drop in filter performance may occur [35]. Recent studies have shown that not only HMW polymers but also hydrophobic beer ingredients affect the filterability [20]. Depending on the used filter materials significant differences in the measured flow rates occurred. These volatiles are not measured within the scope of standard beer analyses, but may cause a drop in filterability.

To investigate whether similar phenomena affect crossflow membrane filtration, fatty acids and MCFA ethyl ester were spiked to beer samples and filtration performance was determined. Furthermore, beer composition in terms of extract, alcohol, viscosity, β-glucan as well as volatiles in course of filtration has been studied. As part of this study not only filtration performance, but also cleanability of the membrane modules was examined. Because CFMF run in several cycles, a complete removal of beer contamination must be guaranteed.

Table 1 Standard analysis of the unfiltered beer samples (n = 3) according to MEBAK [13]

	Unit	Control beer	Beer + MCFA ethyl ester	Beer + fatty acids
Real extract	[mas-%]	3.6 ± 0.1	3.7 ± 0.0	3.6 ± 0.0
pH value		4.3 ± 0.1	4.3 ± 0.0	4.3 ± 0.0
Beer viscosity	[mPa×s]	1.59 ± 0.0	1.58 ± 0.1	1.58 ± 0.1
Turbidity 90°	[EBC]	66.8 **± 4.6**	53.6 ± 18.0	114.5 **± 30.1**
Turbidity 25°	[EBC]	100.2 **± 5.6**	64.9 ± 13.1	138.9 **± 27.6**

2 Material and methods

2.1 Sample preparation

For the filtration experiments, a bottom fermented Pilsner beer of a German brewery was used, which was drawn directly from the storage tank. The general composition of control beer sample is shown in table 1. For the investigation of filtration influences of fatty acids as well as MCFA ethyl esters, 50 l control beer were mixed with 0.8 g/l hop antifoam-agent (Botanix Ldt., Kent), normally used in yeast propagation processes or 0.077 g/l Ethyl hexanoate ($C_8H_{16}O_2$, Fluka 21550; Fluka Analytical, Switzerland) and 0.087 g/l Ethyl octanoate ($C_{10}H_{20}O_2$, Schuchardt OC129; Merck Schuchardt OHG, Germany). Analysis of the hop antifoam resulted in high amounts of (9Z,12Z)-9,12-Octadecadienoic acid ($C_{18}H_{32}O_2$), (9Z,12Z,15Z)-9,12,15-Octadecatrienoic acid ($C_{18}H_{30}O_2$), (9Z)-Octadecenoic acid ($C_{18}H_{34}O_2$). Dosage was carried out directly prior the filtration in order to minimize possible precipitation reactions.

The selection of hydrophobic substances was evaluated on basis of preliminary tests, whereas little effects of ethyl hexanoate but higher ones of ethyl octanoate could be demonstrated in laboratory filtration trials. These yeast metabolites can enter rough beer during fermentation in limited extent. Higher amounts pass into beer via cell lysis. The examined concentrations were adjusted to these preliminary tests. To exclude further cell components (e. g. cell wall) or ingredients (e. g. glycogen) volatiles were added with high purity. Besides yeast, hydrophobic substances can be entered into beer due to the addition of hops. A practical oriented simulation of different hydrophobic hop ingredients could be achieved by the use of the highly purified hop antifoam-agent. The beer composition of the two prepared samples can also be found in table 1.

2.2 Beer filtration

Beer filtration was performed in two different scales. The laboratory membrane filtration was used for characterization of samples. Beer filtration was performed on a CFMF system, wherein not only pressure increase over time, but also membrane cleanability was investigated.

Determination of the filterability

Dead-end filtration was accomplished on an automatic laboratory filter system (see Figure 1) consisting of two stainless steel vessels with cooling jacket and a filter unit for DE as well as membrane filtrations. Pressure and temperature sensor as well as an automatic valve are connected to a controller. The data recording and controlling is carried out with the program Virtual Expert (Gimbo mbH, Freising) considering the variables non-filtrate temperature, filtration pressure and filtrate weight over time [20]. Filterability was determined using the Esser-test, calculating maximum filtrate weight G_{max} (see Eq. 2.1) [8].

$$G_{max}[g] = \frac{t_2 - t_1}{\frac{t_2}{G_2} - \frac{t_1}{G_1}} \qquad \text{(Eq. 2.1)}$$

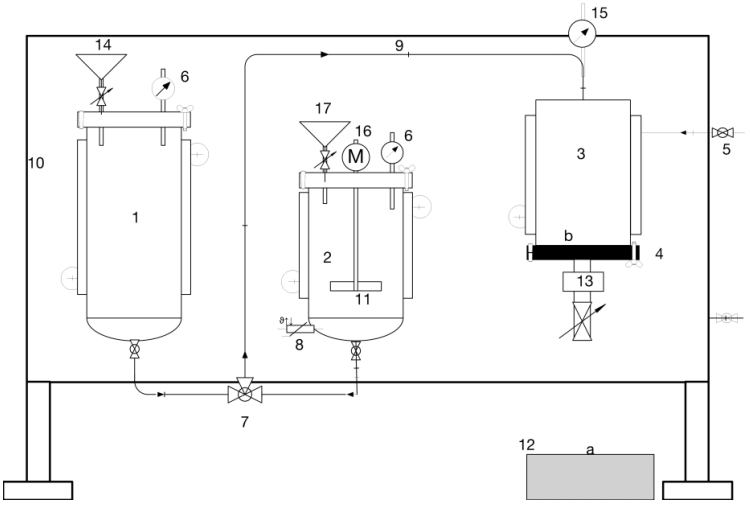

Fig. 1 Automatic laboratory filter system consisting of a precoat vessel (1), a sample vessel (2) and a filter unit (3). A sieve with 15 µm pore size (Raible-test) or a membrane (Esser-test) could be placed (b) in this filter unit. The filter is equipped with a cooling unit (5), a CO_2-connections (6), an automatic valve (7), temperature sensor (8), a balance (12) for recording of the filtrate weight (a), outlet valve (13), filling hopper (14, 17), pressure sensor (15) and an electrical stirrer (16) for the homogenization of filter aids

The sample volume of 200 ml beer tempered to 5 °C was filled into sample vessel and pushed via CO_2-pressure of 1 bar in the filter unit. For the experiment the three different membrane materials polyethersulphone (PES), polyamide (PA) and cellulose nitrate (CN) (Sartorius Stedim Biotech GmbH, Göttingen) with a nominal pore size of 0.45 µm were tested. All filtrations were performed at least in triplicate.

Crossflow Membrane Filtration

CFMF experiments were carried out on the pilot plant BMF-06 CFM-Filter (Pentair X-Flow BV, Enschede, Netherlands) in triplicate. The filter consisting of a 100 l buffer tank with cooling jacket as well as one PES hollow fibre membrane module with a filtration area of 49.01 cm² and a pore size of 0.45 µm. The inner diameter of membranes is 1.5 mm. Filtration runs were executed with a constant flow of 3.6 l/h recording pressure changes at P_{inlet}, P_{outlet} and $P_{filtrate}$. The calculation of trans-membrane pressure (*TMP*) (see Eq. 2.2) as well as trans-membrane pressure rise (*TMP-rise*) (see Eq. 2.3) was conducted considering the recorded pressure data.

$$\boldsymbol{TMP = \frac{P_{inlet}+P_{outlet}}{2} - P_{filtrate}} \quad \text{(Eq. 2.2)}$$

$$\boldsymbol{TMP - rise = \frac{TMP_{n+1}}{TMP_{n=0}} - 1} \quad \text{(Eq. 2.3)}$$

After filtration of 50 l beer membranes were removed from filter and subjected to a cleaning. Membrane modules were installed into the cleaning system T/RX-300 (Pentair X-flow BV, Enschede). This system has three pressure sensors for determination of water flow and constant adjustment. At the beginning of cleaning, membranes were rinsed for 5 min against filtration direction with distilled water. Thereafter an alkaline cleaning with sodium hydroxide (1 %) and a followed flushing with water was done. Subsequently an oxidative cleaning for 24 h in 4 l distilled water containing 12 g active chlorine, 4 g Synflux 10 and 4 g Synflux BR 300 (Pentair X-flow BV, Enschede) and an further flushing with water were the last steps. The clean-water-flux (*J*) measurement was performed according to equation 2.4. Membranes were installed in the T/RX-300 and pressure was set at 1 bar. Flow time of 1 l water through the membrane was measured. This clean-water-flux was checked before and after filtration as well as after cleaning. With a flux decline smaller 5 %, membranes were re-used for filtration. Higher deviations resulted in a repetition of cleaning.

$$\boldsymbol{J_{20\,°C} = \frac{Q_P}{A_m} \times \frac{TMP}{TCF}} \quad \text{(Eq. 2.4)}$$

$$\boldsymbol{TCF[20\,°C] = \frac{0.998}{1.794-(0.055\times T[°C])+(0.00076\times T^2[°C])}} \quad \text{(Eq. 2.5)}$$

2.3 Analyses

Standard analysis of beer samples

Standard beer analysis turbidity (MEBAK 2.15.1.2), viscosity (MEBAK 2.28), alcohol, residual extract (MEBAK 2.10.) and pH-value (MEBAK 2.14.) were performed in triplicate according to MEBAK methods [13].

β-Glucan content

The β-glucan contents of unfiltered and filtered beer samples were determined using fluorometric (MEBAK 2.5) and colorimetric multiwell assay. Both methods were calibrated with a 7-point calibration curve by means of SBL β-glucan calibration standard (Scandinavian Brewery Laboratory Ltd., Copenhagen) containing an amount of 500 mg/l. Initially 15 µl standard was transferred in a 96-well plate by means of pipetting robot BioTek Precision XS (BioTek Instruments, Inc., Winooski United States).

For fluorometric measurement 300 µl dye solution containing 5 ml Calcofluor (Sigma Aldrich, Germany) and 495 ml degassed Tris-HCl buffer (0.1 mol/l pH 8.0) were pipetted into each cavity of a 96-well plate. Fluorescence intensity was recorded at an excitation wavelength of 360 nm and a measurement at 445 nm using BioTek synergy H4 (BioTek Instruments, Inc., Winooski United States). For the calculation of glucan content of the samples, a second order non-linear regression curve converting fluorescence intensity in dependence to β-glucan concentration of the 7-point calibration curve was created. Samples were prepared and measured in same way as calibration standards.

Colorimetric method was carried out with 50 mg Congo red dye (Sigma Aldrich, Germany) in 500 ml degassed Tris-HCl buffer (0.1 mol/l pH 8.0) [3]. The detection of colour reaction occurred at 550 nm. Further procedures and result calculation were done in accordance to the fluorometric method.

Flavour substances

The volatiles were detected with a semi-quantitative method using headspace-solid phase microextraction (SPME), permitting the representation of changes in a test series. Assignment of the volatiles on the GC-FID system was confirmed with a GC-MS

system, using linear retention indices [34]. The retention indices in the beer samples were calculated according to equation 2.6 in relation to a series of n-alkanes (chain length C_6–C_{28}).

$$RI = 100 \times C_N + 100 \times (RT_X - RT_N)/(RT_{N+1} - RT_N) \quad \text{(eq. 2.6)}$$

The GC-MS-system (HP 6890N-GC; Agilent) was directly coupled to a Sensi-TOF-MS (Five Technologies, Munich) and was equipped with a capillary separation column (J&W Scientific, stationary phase DB5, length 30 m, internal diameter 0.25 mm, film thickness 0.25 µm). As carrier gas helium 1.5 ml/min (at 60 °C) with a split ratio of 1:10 was used. Injector temperature was 250 °C, transfer line temperature 220 °C. GC oven was held at 100 °C for 5 min and programmed at a rate of 5 °C/min to 240 °C. Ion source temperature was 200 °C and ionization energy amounted –70 eV with a mass range of 35–600 amu.

The GC-FID (SiChromat 3; Siemens, Munich) was equipped with a comparable DB5 column (J&W, same dimensions) and an integrator D2500 (Merck-Hitachi, Darmstadt). As carrier gas helium 1 ml/min (at 60 °C) with a split ratio of 1:20 was used. Injector temperature was 250 °C, detector temperature was 250°C. As fuel gas air and hydrogen (each 2 bar) were used. GC oven was held at 100 °C for 5 min and programmed at a rate of 5 °C/min to 250 °C.

For the enrichment of volatiles, 5.3 ± 0.1 g samples were weighted in a 20 ml headspace vials and sealed with an aluminium cap and a septum (Butyl/PTFE, Achroma, Müllheim). After incubation for 30 min at 25 °C with the SPME fibre (Stable Flex Divinylbenzol/ Carboxen/ PDMS 50/30 µm, grey; Supelco, Bellafonte, PA/USA), GC-Analysis was performed.

2.4 Statistics

Statistical analyses were carried out using OriginPro 2015G (OriginLab Cooperation, Northampton, USA). To compare differences in beer composition at different filtration times or filtration performance a one-way ANOVA with Tukey–Kramer multiple comparisons post-test using significance level (α) 0.05 was determined. Furthermore Pearson correlation coefficients (*r*) were determined.

3 Results and discussion

3.1 Filterability

Esser-test was used for the determination of filtration performance of control beer and spiked beer samples. The observed results (see Figure 2) differed with respect to the used membrane material, whereas highest filterability could be measured with CN membranes. PES and PA membranes had comparable G_{max}.

Although sample composition has been varied in a wide range, no significant differences ($P > 0.05$) between the control sample and the sample with MCFA ethyl esters could be measured using CN membranes, but dosage of fatty acids resulted in a significantly ($P < 0.05$) lower filterability. Even control beer samples had best filterability using PES membranes. The fatty acid spiked beer

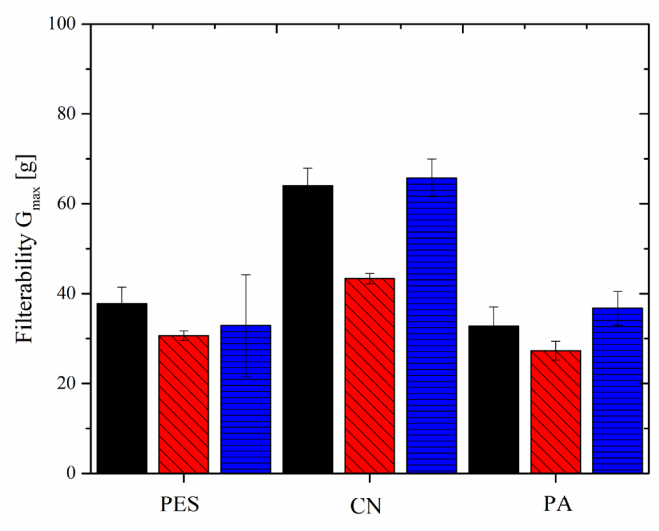

Fig. 2 **Average and standard deviation of filterability G_{max} [g] (n = 10) using cellulose nitrate (CN), polyamid (PA) and polyethersulphone (PES) membranes. legend: ■ control beer, ■ beer + fatty acids, ■ beer + MCFA ethyl esters**

samples showed significantly ($P < 0.05$) lower results compared to control beer, but not in samples with MCFA ethyl esters. Using the PA membranes, no significant differences ($P > 0.05$) in filtration performance could be observed after dosage of fatty acids and MCFA ethyl esters in comparison to the control beer.

3.2 Crossflow membrane filtration

The beer filtration experiments using pilot scale BMF-06 (Pentair X-flow, Enschede) allowed the filtration of 100 l rough beer using PES-membranes. The designed filtration protocol aimed to filter a constant volume of 3.6 l/h beer until a maximum *TMP-rise* of 1.2 bar was achieved, at which a backflush of the membrane was carried out. During the shown experimental series only the beer containing fatty acids reached this maximum pressure. In order to compare filtrations, the initial pressure onto membranes was assessed in relation to further pressure increase, in the following named *TMP-rise*. The results of filtration trials are shown in figure 3.

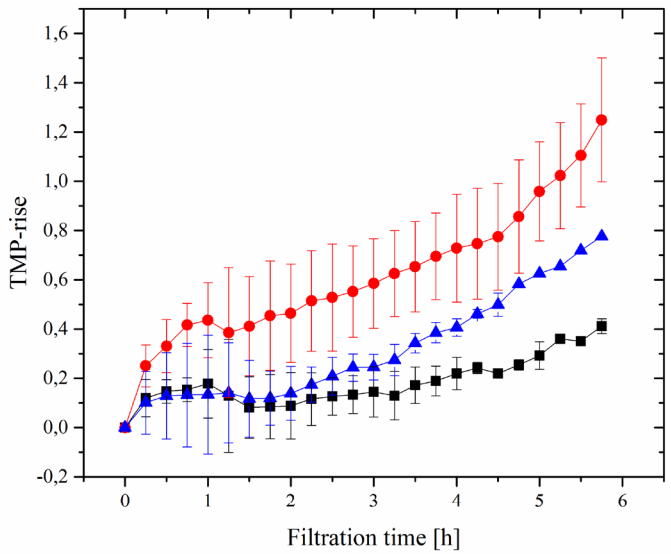

Fig. 3 **Average and standard deviation (n = 3) of trans-membrane pressure (TMP) rise over filtration time. legend: ■ control beer, ▲ beer + MCFA ethyl esters, ● beer+ fatty acids**

Lowest *TMP-rise* could be observed in control beer filtration. Only a slight rise of 0.4 ± 0.03 (n = 3) could be measured in these samples over a filtration period of 6 h. Samples with ethyl hexanoate and octanoate had a primarily increase after second filtration hour and finish with a value of 0.8 ± 0.06 (n = 3). In contrary, samples containing fatty acids had a pressure rise directly after filtration start. A maximum *TMP-rise* of 1.2 ± 0.25 (n = 3) could be measured in these beer samples. Similar to filtration tests in laboratory scale, a decline of filtration performance could be shown because of an addition of hydrophobic substances. A comparison of filterability in laboratory scale and the slope of *TMP* in pilot scale revealed no correlation using PA (r = –0.41, P > 0.05) and CN membranes (r = –0.70, P > 0.05), whereas PES membranes showed a significant connection (r = –0.99, P < 0.05).

Concerning the effects of hydrophobic agents in beer, not only the filtration performance, but also the cleanability of the membranes after filtration process was to investigate. The cleaning condition of the membranes can be determined by help of pure water-flux (J) when comparing fresh and cleaned modules. A flux decline ($J_{20\,°C}$) of –2.8 ± 1.4 % (n = 3) after filtration of control sample could be detected. The samples with fatty acids resulted in a drop of –10.4 ± 6.2 % (n = 3) and the samples with MCFA ethyl esters led to a decrease of -8.1 ± 2.4 % (n = 3). The applied cleaning process was not able to reconvert these membranes into the original state. Similar to the *TMP-rises*, the highest water-flux decrease occurred in the membranes after contact with antifoam spiked beer, followed by the membranes of the aroma compound experiments. The cleaning was repeated in these two experimental series a second time until a flux decline lower than 5 % has been achieved.

A decline of membrane filterability could be shown using model solutions containing β-glucan and different MCFA ethyl esters. Furthermore, a decrease of flavouring substances depending on the chain length of fatty acid residues could be detected [20]. PES-filtration experiments of proteinaceous solutions resulted in similar observation, whereas a flux decline with increasing caprylic acid concentration could be demonstrated [25]. Related influences of hydrophobic substances could be detected in the filtration of waste water [2, 11].

3.3 Beer analysis

The standard traits viscosity, pH-value, alcohol content as well as residual extract (data not shown) decreased during the first filtration hour of CFMF. Thereafter a constant level of the standard composition could be examined. Extract and pH-value showed no significant differences (P > 0.05) within all samples when comparing contents in unfiltered samples and filtered beer over the whole period, whereas the viscosity of control beer exhibited higher values (P < 0.05) than the filtrate after one hour. Samples with dosage of antifoam and MCFA esters showed no significant differences (P > 0.05). At the end of filtration, all beer samples did not differ (P > 0.05) in the examined standard traits in comparison to unfiltered samples. These results clearly demonstrate that the basic beer composition was not affected by crossflow filtration. Furthermore, the β-glucan concentration in rough and filtered beer was determined using a fluorometric staining method (see Figure 4). High concentrations of β-glucans in rough control beer sample (336.6 ± 7.9 mg/l, n = 3×4) could be measured. A decrease to 157.4 ± 15.5 mg/l (n = 3×4) could be observed during the first filtration hour. In further course of CFMF, only small amount of β-glucan was removed, resulting in concentrations in filtrate about 300 mg/l. This drop in β-glucan concentration was also found in samples containing antifoam agent (134.3 ± 41.0 mg/l, n = 3×4) and MCFA ethyl esters (48.3 ± 14.6 mg/l, n = 3×4). In addition, β-glucan contents in these samples had a lower initial concentration. Although, identical initial beer was used for the experiments, only lower levels of β-glucan were measurable in samples with added hydrophobic substances. The investigation of correlations between

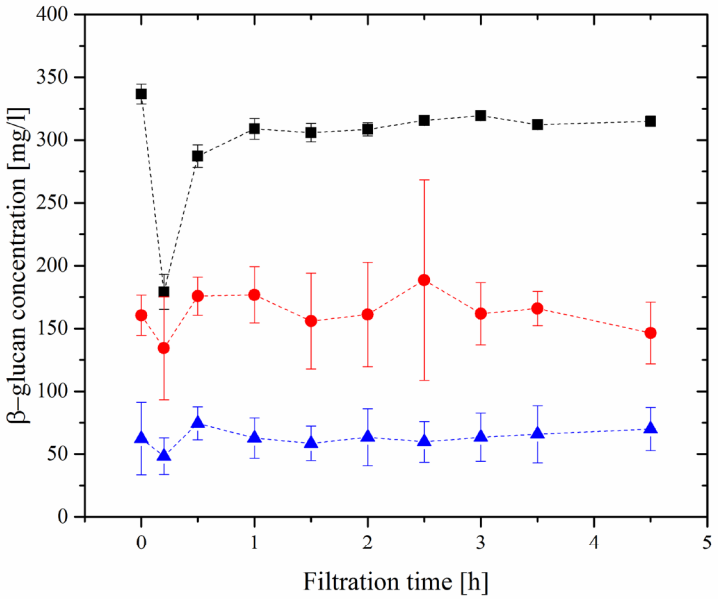

Fig. 4 **β-Glucan concentration (n = 3 × 4) determined using fluorometric assay, legend: ■ control beer, ▲ beer + MCFA ethyl esters, ● beer+ fatty acids**

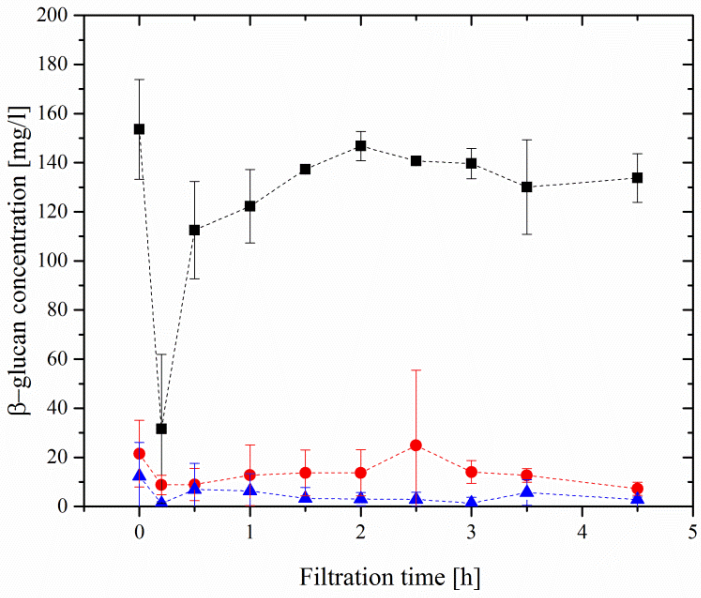

Fig. 5 **β-Glucan concentration (n = 3 × 4) in the course of CFMF determined using colorimetric assay, legend: ■ control beer, ▲ beer + MCFA ethyl esters, ● beer+ fatty acids**

filtration performance and fluorometric β-glucan content resulted in no significant correlations of neither *TMP-rise* ($r = -0.77$, $P > 0.05$) nor the filterability using PES ($r = 0.78$, $P > 0.05$), CN ($r = 0.09$, $P > 0.05$) or PA ($r = -0.26$, $P > 0.05$) membranes.

An additional determination method was performed using colorimetric Congo red assay, since the fluorometric method could not eliminate peculiar doubts concerning the β-glucan concentrations (see Figure 5). This method yielded in similar profiles of β-glucan content, though on lower concentration levels. The *TMP-rise* ($r = -0.93$, $P > 0.05$) as well as the filterability using PES-membranes ($r = 0.93$, $P > 0.05$) showed correlations to colorimetrically determined β-glucan content in rough beer, but not by use of CN ($r = 0.39$, $P > 0.05$) or PA ($r = 0.03$, $P > 0.05$) membranes. For more extensive statistical analysis further investigation would be necessary. The applied Congo red method is described to measure HMW β-glucans [3], whereas a colour reaction of β-glucan with Calcofluor has already been described regarding smaller molecular masses [13, 15]. This difference is supposed to be caused due to variations in dye-β-glucan interactions using Calcofluor-white [38] and Congo red [22]. In general the dyes react via van der Waals forces, ionic interactions and H-bonds with β-glucan molecules [22, 37, 38]. But due to the different molecular structures of the dyes and resulting bonds to polysaccharides great differences in total concentration could be observed.

The measured β-glucan concentration yielded plausible amounts in control beer by means of both methods. Large declines in spiked samples may occur because of measurement error. Another possibility may be the disturbances of colour reactions through the presence of high amounts of hydrophobic substances in beer. Hints on interactions between volatiles and β-glucans can be found in literature [4, 6, 29, 31]. To observe interactions between β-glucan particles and volatiles, further investigations are necessary.

Moreover, the volatiles were analysed in course of CFMF. Identification and quantification were performed investigating retention indices of beer flavour substances on TOF-MS and GC-FID. Figure 6 shows changes in 3-methylbutyl acetate (C7H14O2) content. The control and antifoam samples exhibited only slight modifications during the filtration process. Samples with MCFA ethyl esters decreased about 40 % immediately after filtration start.

The percental changes of flavour substances at the end of filtration are shown in table 2. Contents in control beer declined slightly during filtration process. Especially smaller molecules like 3-methyl-1-butanol ($C_5H_{12}O$) had no decrease caused by membrane filtration. Only ethyl decanoate ($C_{12}H_{24}O_2$) dropped at the end of process to 60 % of initial content. In the control experiments no reduction of flavouring substances with increasing fatty acid chain length could be measured. In beer samples spiked with fatty acids and MCFA ethyl esters, particularly ethyl octanoate ($C_{10}H_{20}O_2$), phenyl ethyl acetate ($C_{10}H_{12}O_2$) and ethyl decanoate showed a high decline.

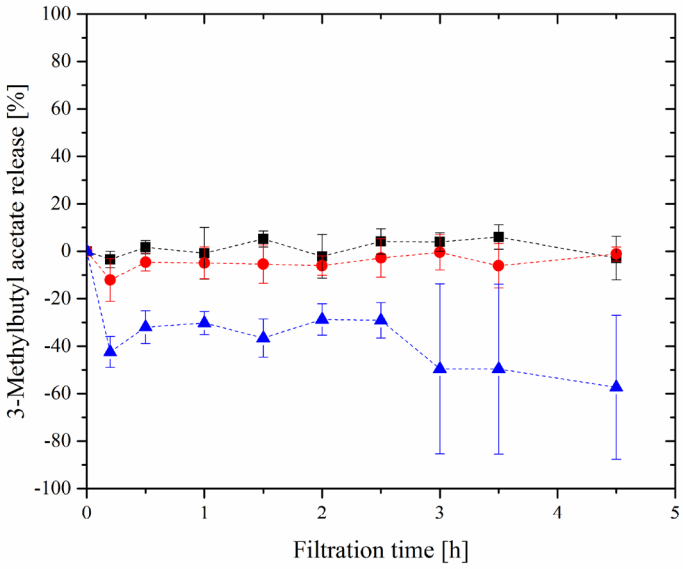

Fig. 6 **Aroma release [%] of 3-Methylbutyl acetate ($n = 3$) in the course of CFMF determined by GC-FID, legend: ■ control beer, ▲ beer + MCFA ethyl esters, ● beer+ fatty acids**

According to *Fritsch* et al. [9] the typical orthonasal beer aroma can be produced by combining 23 odorants using water as matrix. These odorants include not only 3-methyl butanol or (E)-β-damascenone but also ethyl octanoate and ethyl hexanoate, which decreased during the CFMF trials. Furthermore, the authors were able to show the importance of ethyl octanoate in Pilsner beer types because of its high flavour dilution factor of 2048. Changes in beer aroma composition could also be shown by Walla [36] in both DE as well as membrane filtration. The filtration with polysulphone capillary membranes resulted in a drop of MCFA ethyl esters and free fatty acid content with a chain length of C_8–C_{12} in comparison to DE filtration, whereas higher alcohols and acetate esters showed only a slight change [36]. These results were confirmed by Eagles and Wakeman [7], they observed a slight decrease of ethyl acetate during their filtration experiments in beer model. In this context, a decline in filterability as well as a MCFA ethyl ester content with increasing chain length were found [20]. However, these substances do not exhibit a decrease during membrane filtration, rather high concentrations lead to a more rapid blocking and *TMP-rise* of the membranes.

Table 2 **Average percentage decrease of a selection of beer flavour components ($n = 3$) in filtrated beer at end of crossflow filtration**

	Unit	Control beer	Beer + MCFA ethyl esters	Beer + fatty acids
3-Methyl-1-butanol	[%]	55.0	15.1	3.2
3-Methylbutyl acetate	[%]	–2.9	–36.0	–1.2
Ethyl hexanoate	[%]	1.3	16.1	–16.7
Phenyl ethanol	[%]	–0,8	–13.6	–2.9
Ethyl octanoate	[%]	–23.9	–27.0	–30.4
Phenyl ethyl acetate	[%]	16.8	–17.6	–21.9
Ethyl decanoate	[%]	-43.8	-45.2	-60.2

4 Conclusion

Based on the shown results influences of hydrophobic substances like fatty acids and volatiles on the filter performance can be demonstrated in both static as well as dynamic membrane filtration. In this context, high correlations between the laboratory filtration and pilot scale CFMF using 0.45 µm sized PES

membranes were possible. But not only significant impact on pressure rise, but also influences on beer composition could be determined. Although standard beer composition (extract, alcohol) did not change significantly, the β-glucan concentration decreased due to filtration process. Furthermore, the two observed β-glucan methods correlated with different extents to the *TMP-rise* of the pilot system. Therefore influences of HMW β-glucans could be achieved, since these polymers are detected by the colorimetric β-glucan assay [3].

The addition of hydrophobic substances not only resulted in a faster and steeper *TMP-rise*, but also in a decreasing volatile content in beer. In this case, a decline in MCFA ethyl ester as well as acetate ester content could be observed. Based on these results, it could be summarized that HMW β-glucans in combination with hydrophobic substances causes degradation in membrane filterability. Since hydrophobic substances like volatiles are mainly introduced by yeast fermentation and autolysis, a focus on yeast culture, metabolic stress effects and shear forces must be ensured. The exact nature of retention and interaction of polysaccharides and hydrophobic substances cannot be found in literature. For this purpose further experiments must be carried out. In particular, the type of retention on the membrane surfaces as well as interactions with polysaccharides has to be exposed by means of imaging techniques.

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