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Comparative Identification of Filtration-Inhibitory Substances in Membrane and Diatomaceous Earth Filtration of Beer

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„Wo das Latein der Wissenschaft aufhört, fängt das Latein der Kunst an, und die Kunst spielt im Braugewerbe keine kleine Rolle.“

Wilhelm Windisch

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Preface

Michael Kupetz

Dipl.-Ing.

The results and publications of this thesis were developed at the Technische Universität München, Chair of Brewing and Beverage Technology from 2012 to 2016.

Peer-reviewed publications

The following **peer-reviewed publications** (shown in chronological order) were generated in the period of this work:

1. **Kupetz, M., Zarnkow, M., Sacher, B. Becker, T. (2015). "Interactions between Dissolved β-Glucans and Medium Chain Fatty Acid Ethyl Esters in Model Beer Solution and their Impact on the Filterability." Journal of the American Society of Brewing Chemists 73(4): 323-330.**

2. **Kupetz, M., Procopio, S., Sacher, B., Becker, T. (2015). "Critical Review of the Methods of β-Glucan Analysis and its Significance in the Beer Filtration Process." European Food Research and Technology 241(6): 725-736.**

3. **Kupetz, M., Weber, M., Kollmannsberger, H. Sacher, B., Becker, T. (2015). "Impact of Fatty Acids and Medium Chain Fatty Acid Ethyl Esters on the Beer Crossflow Membrane Filtration." Brewing Science 68 (September/October): 122-129.**

4. **Kupetz, M., Sacher, B., Becker, T. (2016). "Impact of Flavouring Substances on the Aggregation Behaviour of Dissolved Barley β-Glucans in a Model Beer." Carbohydrate Polymers. 143 (2016): 204-211.**

5. Kupetz, M., Aumer, J., Harms, D., Zarnkow, M., Sacher, B., Becker, T. (2017). “High-throughput β-glucan analyses and their relationship with beer filterability.” European Food Research and Technology. 243: 341-351.

Contents

Notation

Latin Letters

Symbol	Unit	Meaning/Definition
A	m^2	Filter area
c	kg/m^3	Proportion of solid particles retained in filter medium
K	-	Kozeny constant
k_{FC}	m^3/h	Flow coefficient
Δh	m	Height of porous medium
M_n	g/mol	Number average molar mass
M_W	g/mol	Weight average molar mass
n	-	Exponent for specific retention mechanisms
Δp	Pa	Pressure difference
p_{FB}	Pa	Pressure filter outlet
p_{RB}	Pa	Pressure filter inlet
q	m^3/s	Filtrate flow
R_h	m^{-1}	Filter resistance
r_h	nm	Hydrodynamic radius
$R_{h,1}$	m^{-1}	Internal irreversible resistance
$R_{h,2}$	m^{-1}	Cake resistance
$R_{h,mem}$	m^{-1}	Membrane resistance
r_{rms}	nm	Gyration radius
S_0	m^{-1}	Specific surface area
t	s	Time
$\dot{V}$	m^3/s	Volume flow
$V_{F,A}$	m^3	Filtered volume
x_r^2	-	Aggregation number

Greek Letters

Symbol	Unit	Meaning/Definition
α	m^{-2}	Filter resistance
α_S	m^{-2}	Specific filter cake resistance
β_0	m^{-1}	Resistance of precoat layer
ε	-	Porosity
υ	-	r_{rms} confirmation plot (exponent from the plot r_{rms} versus Mw)
η_L	Pa·s	Viscosity of liquid
ρ_S	kg/m^3	Particle density
$\varphi_{F,A}$	kg/m^3	Solid content filter aid

Abbreviations

Symbol	Meaning/Definition
α-Al_2O_3	Alpha-aluminium oxide
BT-F	Bright beer tank
BT-R	Unfiltered beer buffer tank
C_{xx}	Number of carbon atoms in fatty acids
CLSM	Confocal laser scanning microscopy
C/S	Centrifuge/separator
DE	Diatomaceous earth
EBC	European Brewing Convention
F	Precoat filtration
FA-D	Filter aid dosage
FF	Final filtration
FM	Membrane filtration
HCl	Hydrogen chloride
HV	Signal amplification

log K_{OW}/ log P	Partition coefficient
$MgSO_4$	Magnesium sulphate
MCFA	Medium chain fatty acid
Na_2CO_3	Sodium carbonate
PES	Polyethersulphone
S	Stabilization
SC	Continuous stabilization
SM-D	Stabilizer dosage
Tris	Tris(hydroxymethyl)aminomethane
ZrO_2	Zirconium-(IV) oxide

1 Summary

Requirements regarding the aroma stability and shelf life of food and beverages are increasingly in the focus of consumers. For the brewing industry, this represents new challenges with respect to production processes and the storage and distribution of beer. To fix the material composition and to increase colloidal stability as well as aroma consistency it is essential to remove haze particles like protein-polyphenol associations or polysaccharides like β-glucans as well as microorganisms like yeast or beer spoilage bacteria at the end of fermentation. For this reason, several precoat and membrane filtration systems have been developed in the past.

The removal of these substances is based on surface and depth filtration effects with different filter media. As a result of adsorption inside the filter materials, haze particles smaller than the pore sizes are removed, resulting in a clogging of filter pores as well as a pressure rise at the filter inlet. In this thesis, filtration-inhibiting substances will be investigated with the main focus on β-glucan participation in membrane and diatomaceous earth (DE) filtration. Therefore, the impact of polymer structure and the origin of β-glucans (derived from yeast or barley) as well as the influence of additional beer ingredients will be examined in more detail.

The connection between the molecular structure of β-glucans and filterability could be observed in membranes and DE filtration. Besides a smaller molar mass, β-1,3;1,6-glycosidic bond glucans from yeast cell walls resulted in a high degradation of membrane (−95%) and DE filtration performance (−90%). Furthermore, interactions between barley β-glucans and volatiles, more precisely medium chain fatty acid ethyl esters from yeast fermentation, could be found. In comparison to pure barley β-glucan samples, the addition of volatiles resulted in a 65% drop in membrane filterability accompanied by a decrease of ethyl octanoate (−58%), ethyl decanoate (−87%) and ethyl dodecanoate (−94%). In addition to an influence on β-glucan agglomeration, interactions of volatiles with membrane material could be identified using locally-resolved image analysis. Although decreased filterability was observed during DE precoat filtration, different effects on filter clogging could be identified with the different substances tested.

In summary, not only β-glucan concentration and molar mass of the cereal β-glucans and thus the malt composition but also the yeast viability and the associated entry of MCFA esters and yeast β-glucan have an important impact on beer filterability.

Zusammenfassung

Die Anforderungen an Aromastabilität und Haltbarkeit von Lebensmitteln als auch Getränken rücken immer mehr in den Fokus der Konsumenten. Dies stellt vor allen Brauereien vor neue Herausforderungen hinsichtlich Produktionsverfahren, Lagerung und Vertrieb des Bieres. Zur Fixierung der stofflichen Zusammensetzung sowie Erhöhung der kolloidalen Stabilität und Aromakonsistenz ist es aus diesem Grund unabdingbar, Trübungsbildner wie Eiweiß-Gerbstoffverbindungen oder Polysaccharide wie β-Glucane, sowie Mikroorganismen wie Hefen und bierverderbende Bakterien am Ende der Gärung aus dem Getränk zu entfernen. Dazu wurden in den letzten Jahren verschiedene Methoden der Anschwemm- und Membranfiltration entwickelt, um einen einwandfreien Geschmack sowie eine Glanzfeinheit der Produkte zu erreichen.

Die Entfernung dieser Stoffe während der Bierfiltration beruht auf Oberflächen- und Tiefenfiltrationseffekten mit verschiedenen Filtermedien. Durch Absorption in der Tiefe dieser Filtermaterialien können Trübungsbildner, die kleiner als die Porengröße sind, zurückgehalten werden, was im Laufe der Filtration zu einer Verblockung der Filterporen sowie zu einer Druckerhöhung auf Retentatseite führen kann. Aus diesem Grund sollte in der aktuellen Arbeit der Einfluss filtrationshemmender Stoffe mit einem Schwerpunkt auf der Beteiligung von β-Glucanen bei der Membran- und Kieselgurfiltration untersucht werden. Hierbei wurde nicht nur die Polymerstruktur und Herkunft der β-Glucane aus Hefe oder Gerste, sondern gleichwohl der Einfluss weiterer Bierinhaltsstoffe erfasst.

In diesem Zusammenhang konnte ein großer Einfluss der β-Glucanstruktur auf die Membran- und Kieselgurfiltration gezeigt werden. Trotz einer geringeren molaren Masse als Gersten-β-Glucane resultierten die β-1,3;1,6-glycosidisch gebundenen Glucane der Hefezellwand in einer stärkeren Reduzierung der Filterleistung bei Membran- (−95 %) und Kieselgurfiltration (−90 %). Weiterhin wurden Interaktionen der β-Glucane mit Aromastoffen, genauer mittelkettigen Fettsäureethylestern (MCFA Ethylester), aus der Gärung festgestellt. Im Vergleich zu reinen Gersten-β-Glucan-Lösungen hatte die Zugabe der Aromastoffe eine Abnahme der Membranfilterleistung um bis zu 65 % zur Folge. Dies wurde begleitet von einer Reduzierung der Aromastoffe Ethyloctanoat (−58 %), Ethyldecanoat (−87 %) und Ethyldodecanoat (−94 %). Neben

einer Erhöhung der β-Glucan Agglomeration, konnten Interaktionen der Ester mit dem Membranmaterial mit Hilfe einer ortsaufgelösten bildgebenden Methode nachgewiesen werden. Wenngleich eine Auswirkung auf die Filterleistung auch bei der Kieselgur-Anschwemmfiltration feststellbar war, so konnten unterschiedliche Effekte an der Beteiligung der untersuchten Inhaltsstoffe an der Filterverblockung ermittelt werden.

Zusammenfassend ergab sich, dass nicht nur die β-Glucankonzentration und molare Masse der zerealen β-Glucane und damit die Malzzusammensetzung sondern auch die Hefeviabilität und der damit einhergehende Eintrag von MCFA Estern und Hefe-β-Glucan entscheidenden Einfluss auf die Filtrierbarkeit des Bieres besitzen.

2 Introduction and motivation

The German beer market has been exposed to a significant recession in recent years. Besides decreased beer production of nearly 20 million hectolitres in the past 20 years, per head consumption of beer has fallen by about 25%. Nevertheless, German beer has gained popularity abroad, which can be seen in export increases of nearly 10% in the same period of time. Thus, exports accounted for an important proportion of total beer production of 16.6% in 2015 [1]. Furthermore, a shift from draft to bottled beer could be observed all over the world [2]. In this context, it could be shown that bottled beer reached distribution distances of more than 210 km in Germany [3].

These changes challenge beer production to completely new demands in terms of stability of taste and appearance. Consumers expect star-bright products, which are durable regarding their composition in foam, flavour and haze months after manufacture. In the brewing industry shelf lives of 6 months to 1 year are now common [2]. For a detailed differentiation of influencing factors 5 different stabilities including foam, colour, haze, flavour and microbiology can be examined. Due to chemical reactions, e.g. the presence of oxygen, environmental factors like heat, or aroma losses, the flavour, bitterness and body of fresh beer may change significantly [4,5]. More important for appreciation of a beer is microbiological and colloidal stability, mainly recognizable due to haze particles found in the beverage. This feature is quickly recognized by untrained beer drinkers and associated with spoilage of the product. In order to decelerate the precipitation of various beer ingredients over time, brewers can apply different types of filtration and stabilization to fix the material composition. This complex haze can consist of microorganisms and their metabolites, as well as components of the raw materials malt, hops and water [6]. In order to control processes and make predictions related to durability, limit values for the presence of microorganisms (0 cells), particularly for yeast (0 cells), and the remaining haze particles were prepared. According to Analytica-EBC [7] brilliant beer is distinguished by a turbidity smaller than 0.5 EBC using light scattering analysis at an angle of 90° [8]. This represents a complex task for filtration processes in order to match requirements for turbidity and shelf life. Over the years, various methods have been developed which aim to optimize the filtration process and reduce costs in beer production [9]. Furthermore, aspects in connection with filter aid disposal have

appeared in recent years and aid brewers constantly faced with problems [10]. To ensure the described requirements and reduce environmental impacts, new filtration methods have been developed which are based on different procedural basics like dead-end and crossflow filtration.

2.1 Filtration basics

Generally, filtrations can be distinguished according to their applied flow direction between dead-end and crossflow process procedures, where dead-end filtration describes a method based on pressure differences between rough and pure medium and vertical flow directions to filter media. In contrast, crossflow filtration is characterized by an additional parallel movement of the rough media along the filter surface [11].

Regardless of place and flow direction of particle retention, filtrations can be described by Darcy's law (see Equation 2-1), which is a basic application for the change in volume flow (q) in dependence on filter area (A), viscosity of the filtered medium (η_L), filter resistance (R_h) and pressure difference (Δp) [12,13]. Because particle retention is influenced by different filtration operations, the mechanical effect of pore or capillary flow caused by driving forces must be considered to overcome a flow resistance for the fluid phase (see Equation 2-2) [14]. Depending on the location of particle retention an increase in filter resistance can be observed [12].

$$q = \frac{\partial V_{F,A}}{\partial t} = \frac{A \cdot \Delta p}{\eta_L \cdot R_h} \qquad \textbf{(2-1)}$$

$$\frac{\partial^2 t}{\partial V_{F,A}^2} = k_{FC} \left(\frac{\partial t}{\partial V_{F,A}} \right)^n \qquad \textbf{(2-2)}$$

Thus, Equation 2-2 describes the dependence on filtered volume ($V_{F,A}$) over time (t), filter surface (A) and flow coefficient (k_{FC}). Flow coefficient is crucial for determining the liquid flow-through amount, which is dependent on layer thickness, type and structure of filter medium, flow properties of liquid and pressure difference (Δp) [12]. Exponent n assumes different values to specific retention mechanisms in operation or changes in the internal structure of the filtering layer. A distinction of the formulas can be determined with regard to process design on pressure or volume flow [15]. In the

food industry, filtration processes often operate at constant filtrate volume flow, in order to ensure production scheduling.

Due to occurring retention effects, various model concepts were developed illustrating these different filtration operations. A fundamental distinction is made between surface and depth filtration [16]. Furthermore, cake, sieve and crossflow filtration can be named as special cases and connections between surface and depth filtration [11,17]. Simplified mathematical model conceptions on filtration processes are shown in Figure 2-1, which have been developed to predict the effectiveness and process performance of applied practical filtrations [18].

2.1.1 Depth filtration

During depth filtration, most separation takes place inside the filter media. Particle removal from unfiltered media is effected by the flow of a suspension through a medium composed of granular or fibrous nature [19]. A substantial proportion of solid particles (c, compare Figure 2-1) that might pass through because of their geometric size are retained in the filter media [17]. This deposition in the interior of the filter causes an accumulation of deposited particles within the medium, which results in continuous changes to the filter media structure and affects the rate and flow resistance of filtration [20]. Furthermore, surface blockages of filter material must be avoided to ensure the maintenance of the filtration process.

Particle retention is achieved by means of holding by adhesive forces influenced by various transport mechanisms inside the filter like sieving, interception, inertia, sedimentation, diffusion, charge interactions or hydrodynamic interactions [11]. Regarding equation 2-2, depth filtration can be described using an exponent n between 0–2, where 1 describes an intermediate blocking and 3/2 a standard blocking procedure [12,15].

2.1.2 Surface filtration

In contrast, surface filtration is effected by mechanical particle separation on the surface of a filter media. Due to retained particle properties and flow direction three types can be distinguished [16].

Sieve or blockage filtration describes a process whereby solid particles are retained on the filter media surface because of their geometric size. This is influenced by an exponential pressure rise (p, compare Figure 2-1) at a constant volume flow ($\dot{V}$). Complete blocking can be described with equation 2-2 using an exponent of 2 [11].

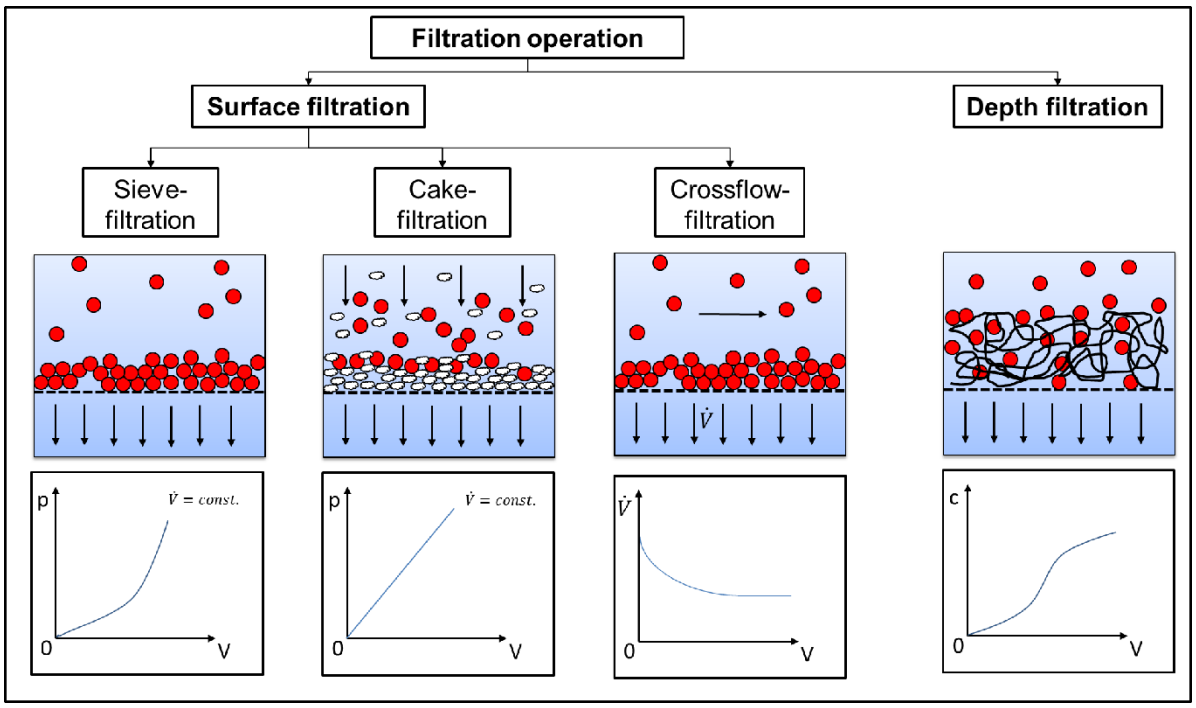

Figure 2-1: Model conceptions on filtration operations, modified according to [11,17,16]. A general distinction can be made between surface and depth filtration. Depth filtration is marked by an increase in solid particles (c) with rising volume (V), due to an exhaustion of absorption capacity of the filter material. Sieve and cake filtration are distinguished via a characteristic pressure rise (p) at a constant volume flow ($\dot{V}$). In the case of crossflow filtration, initially a reduction in volume flow ($\dot{V}$) due to an accumulation of solids on the filter material can be observed, followed by a stationary phase with nearly no change in filtered volume [17].

Cake filtration is a case of surface filtration where solids are retained on the filter media surface with the help of filter aids (compare Figure 2-1) [17]. The filtered volume is influenced by filter cake height, filter area, dynamic fluid viscosity and resulting dynamic filter resistance [11,17]. Retention at the beginning of filtration is determined by the filter media pore size. Over time finer solid particles can be removed from suspension because of sufficiently high loading of suspended particles and filter aids, followed by a “bridging” across the filter pores. Ideally, filter cake resistance increases

in proportion to its thickness, resulting in a constant flow rate ($\dot{V}$). The surface filtration with constant pressure rise could be described using the exponent 0 [11].

Crossflow filtration is a further feature of surface filtration, where a crossflow suppresses the formation of a filter cake on filter media (compare Figure 2-1). Total exemption of particles on filter media surface cannot be guaranteed, which is why a stationary particle layer is desired [16]. As a result of a pressure difference (transmembrane pressure) between the rough and pure side, permeate is removed from the filter and retentate is further circulated [16,17]. This mechanism leads to a concentration of retentate and can be performed as long as the liquid remains pumpable [11].

Surface and depth filtration provide process engineering basics for diatomaceous earth (DE) and membrane filtration, which are mostly applied in the brewing industry. These types of filtration are mainly distinguished by their filter plants as well as the usage of different filter media (see Figure 2-2). Besides filter equipment, process management as well as filterability of beer have a great impact on beer filtration. Because of this multiplicity of influencing factors, beer filtration will be considered in more detail in the next chapters.

2.2 Beer filtration

The basic approach to beer filtration has not changed since the 1950s, when the diatomaceous earth (DE) gained its importance in Germany [18]. Today, DE is still the most popular filter aid to filter beer all over the world. However, different filter media, filter plants and thus process management systems are applied in the brewing industry, something that became necessary because of different company sizes, beer volumes and required flexibility.

Fundamentally, beer filtration can be performed as batch or continuous process steps. Furthermore, process management can vary because of production scheduling, beer types and volumes as well as different procedural problems like the prevention of pressure shocks. Used filter plants differ, in particular, due to their capacity, geometry and size as well as buffer tanks and pumps before the filter. Furthermore, filter media can vary between filter aids with different particle sizes like DE or perlite and membrane

materials (e.g. polyethersulphone) with various pore sizes. In addition, beer composition has a considerable influence on filter performance (see Figure 2-2) [6]. Thus, each process step during malting and brewing has an influence on the ultimate filterability. In order to be as flexible as possible due to varying beer filterability, precoat filtration has become more and more established over the years in the brewing industry.

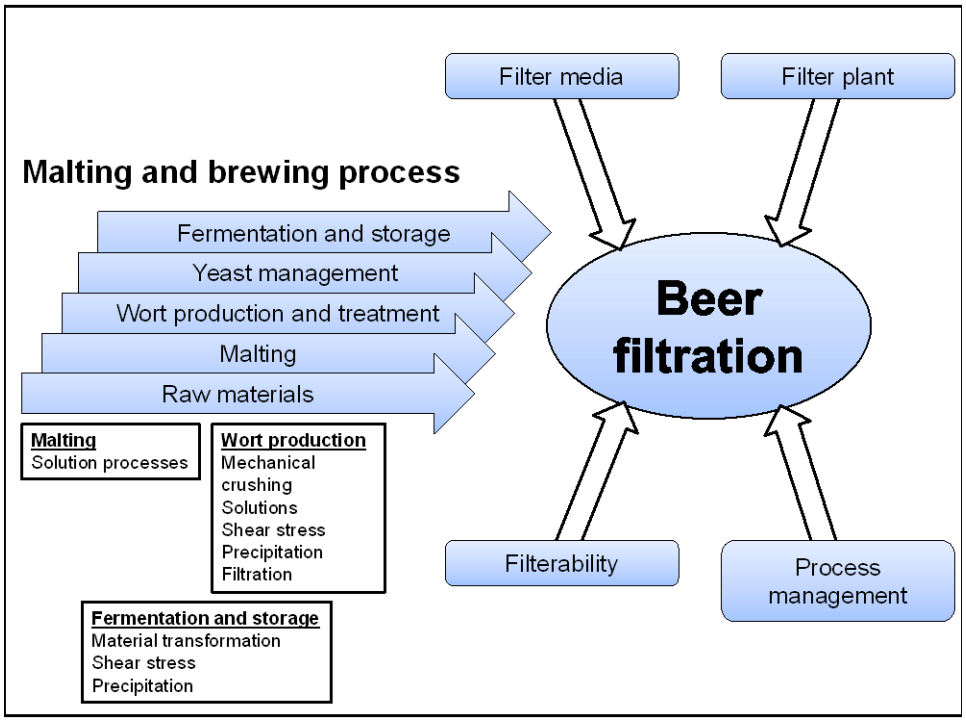

Figure 2-2: Influencing factors on beer filtration. Besides filter plant (e.g. filter type or capacity) and different used filter media (e.g. filter aids or membrane materials), process management (e.g. planning of daily batch sizes) influences beer filtration. Furthermore, filterability of beer and thus all production steps during malting and brewing have an impact on filter performance of the beverage [6,21].

2.2.1 Precoat filtration

In a brewery, precoat filtration is applied in three different steps. Firstly, a thin protective layer of filter aid (coarse precoat) is washed on the filter medium. Secondly, a further layer of finer filter aid is applied to the coarse cake in order to ensure the separation of fine particles even at the beginning of the filtration. Finally, smaller amounts of filter aid are added to the unfiltered (rough) liquid, known as body feed. This forms a continuously growing incompressible filter cake, which is capable of maintaining a high permeability and thus a high beer flow [22,23].

Precoat filtration is generally done as a batch process. Usually first stage is performed as a filtration step, whereas the second step is used as polishing filtration [23]. A typical structure of a filter cellar for precoat filtration is shown in Figure 2-3. In addition to the filter unit and some buffer tanks, a centrifuge and an additional filter for the implementation of stabilization can be present. To homogenize the filter aid a mixing tank is built before the filter. For the dosage of filter aid the entry of oxygen must be avoided.

It is nowadays also common to attach a sterile filter for cold sterilization before bottling. A further possibility to increase microbiological product safety would be heat treatment using pasteurization. In addition, units for the carbonation of the beer are located before bottling, but have been omitted in Figure 2-3. Different constructions can be used as filter unit for precoat filtration. Most common are frame, candle and horizontal pressure leaf filters [24,25]. These filter designs differ because of filter media (e.g. cartridge or flat metal sieve) to which filter aids are applied, loading capacity and thus filter volume as well as flexibility in production of different batch sizes.

In addition to the filter units, sensors for turbidity and pressure measurement are used for the evaluation of the filtration process. Maintenance and control of beer haze during precoat filtration is performed using turbidity measurement at filter inlet and outlet at a 90° angle detecting particles smaller than 1 µm. Furthermore, a pressure rise at the filter inlet provides information regarding particle retention and filter cake composition. Excessive increases in pressure can be controlled by body feed composition [23]. **Dosage of filter aid** for precoating occurs as a suspension in degassed water with a mixing ratio of H_2O to filter aid of 5:1. A mixing time of 10–30 min and gassing with carbon dioxide permits the expulsion of oxygen [23,24]. The amount of filter aid dosage and composition is still based on experience values, whereby automation using turbidity measurement at filter inlet, filtrate flow and pressure difference has been applied in some breweries [24].

An efficient and economical filter aid is marked by rigid, intricately shaped and individual particles, can form highly permeable, nearly incompressible filter cakes, remove even the finest solids at high flow rates and must be chemically inert and essentially insoluble in the liquid being filtered [22]. The selection of filter aid composition, amounts and mixing grades should result in an average of high clarity

effects and low pressure rises [22,23]. In this context DE has been established as an effective filter aid for beer clarification because of its high internal porosity [18,27,28].

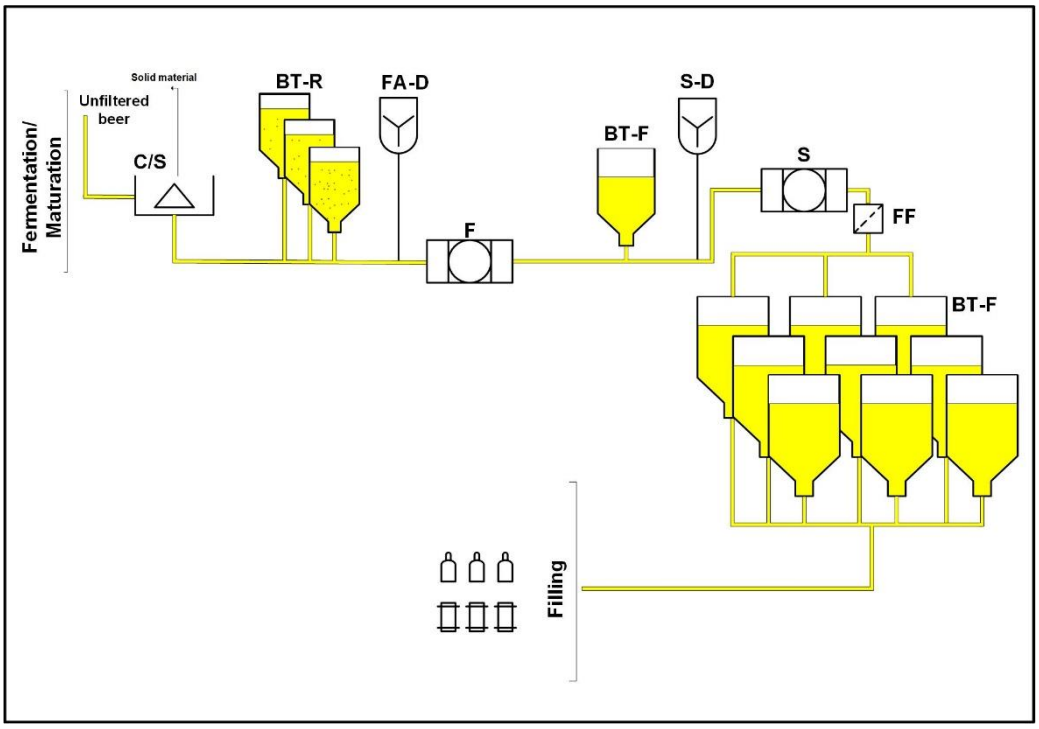

Figure 2-3: Precoat filter system modified according to Bellmer [26]. The filter cellar could contain a centrifuge or separator (C/S) for preliminary clarification of beer as well as the removal of yeast cells and large trub particles. An unfiltered beer buffer tank (BT-R) is used for safe production and pressure-impulse-free filling of filter units. A stirring vessel for filter aid dosage (FA-D) serves the homogeneous dosage of filter aids into beer. Subsequently, the mixture of beer and filter aids is washed on the precoat filter (F), where solid and liquid components are separated. After filtration, beer stabilization could be connected, which compromises a stabilizer dosage (SM-D) and stabilization filter unit (S). This beer stabilization serves to remove proteins and polyphenols to increase the chemical and physical stability. Stabilization in the brewing industry is often performed using a cartridge filter. Furthermore, bright beer tanks (BT-F) and final filtration (FF) can be found before bottling [23].

Diatomaceous earth or kieselguhr consists of three-dimensional exoskeletons of freshwater or seawater organisms. High levels of purity and variety in size and shape are ensured thanks to their location on the ocean floor over millions of years [22]. Deposits of DE are mined in France, the United States of America and Russia. Manufacturing is marked by several thermal processes to remove water and organic impurities and screening by particle diameter. Body feed grades (medium permeability: 0.8 Darcy) have an average particle size of 7–20 µm, a brown or pink colour and

appear like original diatoms [26]. Calcination for the reduction of any organic debris is performed to increase the purity at 800–1000°C [23]. 91% of DE consists of SiO_2 with a remaining proportion of salts from aluminium, iron and calcium [18]. Flux-calcined partially fused DE is used for the first precoating. The amorphous pieces contain 88% SiO_2 and have particle sizes bigger than 20 µm. Sintering of DE particles is performed at 1000–1200°C with the addition of Na_2CO_3 [23]. This results in larger, more complex particles with faster flow rate and higher permeability.

In general, 0.75–2.0 kg/m^2 filter area DE is used for beer filtration [23]. This amount is divided into a dosage of flux-calcined DE (200–700 g/m^2) for first precoating and a second dosage of finer filter aid (400–800 g/m^2) to increase particle retention of the precoat layer already at the beginning of the filtration. Afterwards an average body feed of 80 g/hl (50–150 g/hl) fine DE is used for constant formation of filter cake. Due to filter cake composition with various DE particle sizes, a minimum haze particle cut-off size of 0.4–0.5 µm can be observed [18]. Disadvantages of DE usage are a required large amount in comparison to the quantity of solids in beer as well as a health risk due to the respirable dry powder [18,23]. Because of an unsolved disposal problem for DE as well as possible health damage, other filter aids were investigated in beer filtration. **Perlite** is an alternative filter aid for beer filtration and consists of volcanic rock comprised of silicates from aluminium, potassium and sodium. Material is crushed and heated to softening point, which results in an expansion of the volcanic rocks producing a very light material [18]. Thereafter, foamy perlite bubbles are milled and sorted [23]. The resulting filter aid has a permeability of 0.15–6 Darcy and is only used for precoating due to its slow sedimentation properties and poor clarifying assets of fine particles. Because of a lack of internal porosity, low adsorptive properties and a flat smooth surface, filter performance for the manufacturing of bright brilliant beer was not successful [18,23,29]. Furthermore **alternative filtration aids** like cellulose fibres, silica hydrogels used as body feed (stabilizer), Crosspure®, polymer powder or rice hull ash were tested in beer filtration with varying success [30-34]. Besides precoat filtration especially the membrane filtration has gained great popularity in the brewing industry in recent years.

2.2.2 Membrane filtration

During membrane filtration, particle separations are performed in dependence on haze particle size and the pore size of the used filter media. The filter materials are termed membranes and can be differentiated according to geometric sizes, structure (porosity, grain size distribution, pore shape), mechanical, chemical and thermal resistance and surface properties (wettability, zeta potential, adsorption) [35]. Because of these different properties, membrane filtration can be performed as dead-end and crossflow processes. Crossflow filtration has been proven in this context in the brewing industry due to a renouncement of filter aids like DE, less use of manpower and thus a high level of automation, less product losses as well as testable integrity of membranes [36,37].

A typical structure of a membrane filter arrangement in the brewing industry is shown in Figure 2-4. A lower plant-engineering effort in comparison to precoat filtration systems is noticeable. Due to this continuous process design, a continuous stabilization of beer can be carried out during membrane filtration [38]. An application of separation systems (centrifuge) before filtration is optional and depends on variability in beer haze composition. Depending on plant type and supplier, differences in membrane material and design may occur in the food and beverage industry. **Membrane design** can be differentiated into hollow fibre, multi-channel, spiral wound or flat membranes and is influenced by used material [39]. **Choice of filter material** depends on the composition of the unfiltered medium and requirements regarding clarity and durability; thus membrane material is subjected to large variations due to available organic and inorganic materials [36]. Furthermore an easy and complete regeneration must be ensured. To increase membrane stability and filter performance, composite membranes are used which are characterized by a multi-layer structure. This allows higher retention of haze particles and a protection of selective membrane surface. Especially membranes with asymmetric pores have been proven in this context [40,41]. Common materials in the food and brewing industry are mainly organic and ceramic membranes. **Polyethersulphone** (PES) is an organic high-performance material and used by several commercial systems in the brewing industry [29,42,43]. These membranes have pore sizes of 0.45–0.65 µm and are manufactured as hollow fibre or flat sheet modules [36,43]. Due to a low affinity for bio-macromolecules, small

adsorption on membrane surface can be determined [39,41]. Since PES is a hydrophobic material, manufacturers use different additives for enhancing the hydrophilic properties [44]. Thus, PES is marked by a resistance to temperature and broad pH ranges [39]. According to van der Sman et al. [41] these properties have a positive effect in beer filtration.

Ceramic membranes are composite membranes composed of a ceramic body, consisting of a thin layer of α-Al_2O_3 and a separation layer of ZrO_2. These membranes are heat sterilisable and stable in the full pH range. Because of this high membrane stability against pressure and temperature, a long lifetime of approximately 10 years can be achieved. Ceramic membranes have a good cleanability. Selectable pore sizes depend on filtration properties of feed solution [45]. Since this membrane material was not used in the experiments, it is not discussed further.

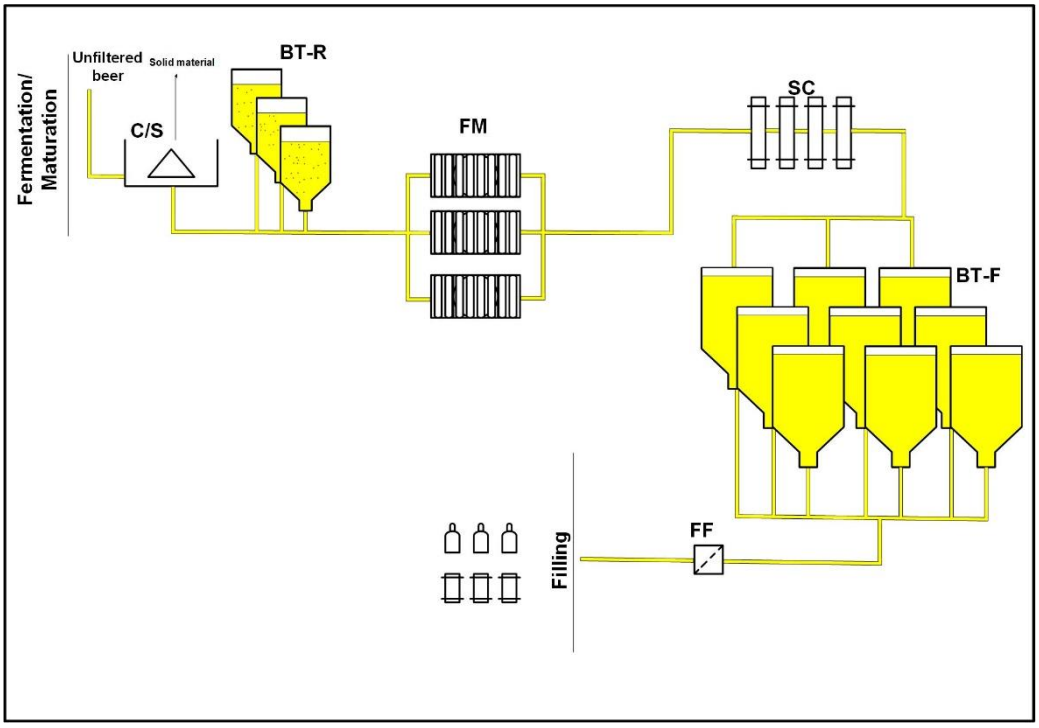

Figure 2-4: Continuous membrane filter system modified according to Gaub [38]. After the fermentation and storage of beer, a centrifuge or separator (C/S) can be used as a first filtration step. Subsequently, beer is collected in unfiltered beer buffer tanks (BT-R) and filtered using different membrane filter systems (FM). In a last step, beer passes continuous stabilization (SC), bright beer tanks (BT-F) and a final filtration (FF) before bottling.

2.2.3 Filter clogging

Regardless of used application, DE and membrane filtration are adversely affected by different **clogging mechanisms**. The kinds of filter clogging and thus degradation in filter performance are influenced by applied filter types as well as filter material characteristics that can be described by Darcy's classical filtration law (see Equation 2-1). Typical filter clogging mechanisms can be differentiated into cake filtration, standard blocking, intermediate blocking and complete blocking, which are shown schematically in Figure 2-5 [46]. Mechanical inhibition due to cake formation is characterized by haze particle sizes much larger than the filter pore size. In contrast, standard blocking occurs by chemical adsorption of particles much smaller than the filter pore size. Furthermore, complete blocking is caused by particles of comparable size to the filter pore, which completely cover pore inlets via mechanical inhibition [41,47]. Such particle adsorptions are largely determined by surface properties of the membrane or filter aid.

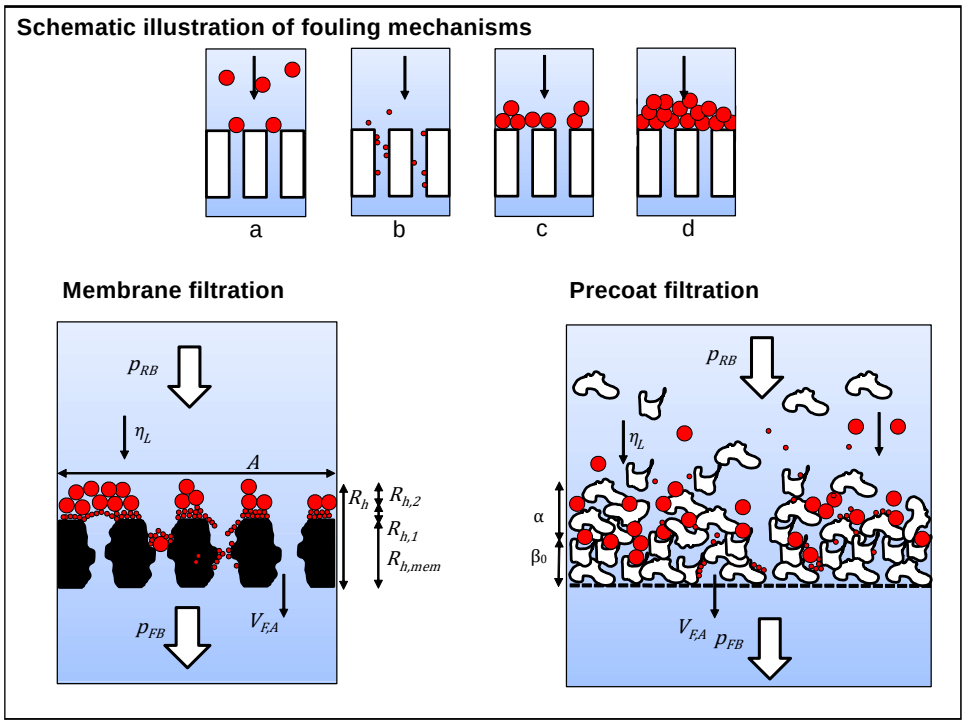

Figure 2-5: Different effects of filter clogging in membrane [47,48] and precoat filtration [15] as well as schematic illustration of four different fouling mechanisms: (a) complete blocking, (b) standard blocking, (c) intermediate blocking and (d) cake filtration according to Wang et al. [49]. Notation: pressure filter outlet (p_{FB}), pressure filter inlet (p_{RB}), liquid viscosity (η_L), filter area (A), filter resistance ($R_{h,mem}$), internal irreversible fouling ($R_{h,1}$), cake resistance ($R_{h,2}$), specific filter cake resistance (α_S), resistance of precoat layer (β_0), filtered volume ($V_{F,A}$).

However, depending on the used filter materials, differences in clogging can be observed which are based on various process engineering principles. Precoat filtration processes can be described with Equation 2-3, known as the Kozeny–Carman equation, where pressure differences arise as a change of driving force between filter inlet (p_{RB}) and outlet (p_{FB}) (see Figure 2-5) [12,15].

$$\Delta p = \dot{V}_{F,A} \cdot \eta_L \cdot (\beta_0 + \alpha \cdot \varphi_{FA} \cdot V_{F,A}) \qquad \textbf{(2-3)}$$

The equation describes permeability through a porous filter cake as a function of pressure rise (Δp) in dependence on liquid viscosity (η_L), resistance of precoat layer (β_0), solid content of the filter aid (φ_{FA}), filtered volume ($V_{F,A}$) and specific filter cake resistance (α_S) [12]. This specific cake resistance is given by Equation 2-4 as a ratio of the empirical Kozeny constant (K), porosity (ε), specific surface area (S_0) and particle density (ρ_s) of the filter aid [50].

$$\alpha_S = K \cdot \frac{S_0^2 \cdot (1-\varepsilon)}{\rho_S \cdot \varepsilon^3} \qquad \textbf{(2-4)}$$

In comparison, the retention of particles during membrane filtration is affected by membrane material, its surface properties as well as its depth and pore structure. Mechanisms of pressure rise due to the influence of different particles can be described using Darcy's equation (see Equation 2-5) [47,51].

$$\Delta p = \frac{q(t) \cdot R_h \cdot \eta_L}{A} = \frac{q(t) \cdot (R_{h,mem} + R_{h,1} + R_{h,2}) \cdot \eta_L}{A} \qquad \textbf{(2-5)}$$

This equation describes pressure rise as a function of permeate flow ($q(t)$), filter resistance (R_h) and medium viscosity (η_L) depending on membrane surface (A). Total filtration resistance does not differentiate between separation locations in the filter membrane. Thus, filter resistance could be summed up in membrane ($R_{h,mem}$), internal irreversible fouling ($R_{h,1}$) and cake resistance ($R_{h,2}$) (see Figure 2-5) [51]. Change of resistance over duration of filtration is affected by particle characteristics like geometry, concentration, interactions among particles as well as filter material characteristics.
Deposition of haze particles in the filter cake or membrane are influenced by size distribution, shape and packing status of the filter aid or cake formation on the membrane. Thus, different beer ingredients have an impact on cake formation, final porosity and permeability of filter cake or membrane. These ingredients occur in beer

in a large variation with respect to origin, size (diameter) and shape and are known as filtration-inhibiting substances that are influencing filterability of beer.

2.3 Filtration-inhibiting substances and haze particles in beer

The complexity of beer is determined by a mixture of cells, aggregates, colloids and macromolecules (compare Figure 2-6) [47]. Thus, particle size and character of beer haze particles range widely. Because beer is stored cold before filling after weeks, the quantity of yeast is insignificant (~6 µm) and the majority of filterable solids range between smaller 0.1 and 5 µm [18,41]. The distribution of filtration-inhibiting substances was described by Kreisz [6], introducing a distinction between ingredients from the raw materials malt, water and hops on the one hand and yeast or microorganisms and their metabolites on the other. Figure 2-6 shows a distinction of substance groups in dependence on size and origin.

Filtration-inhibiting substance groups like proteins, polyphenols and polysaccharides generally get into beer during mashing and boiling processes from raw materials.

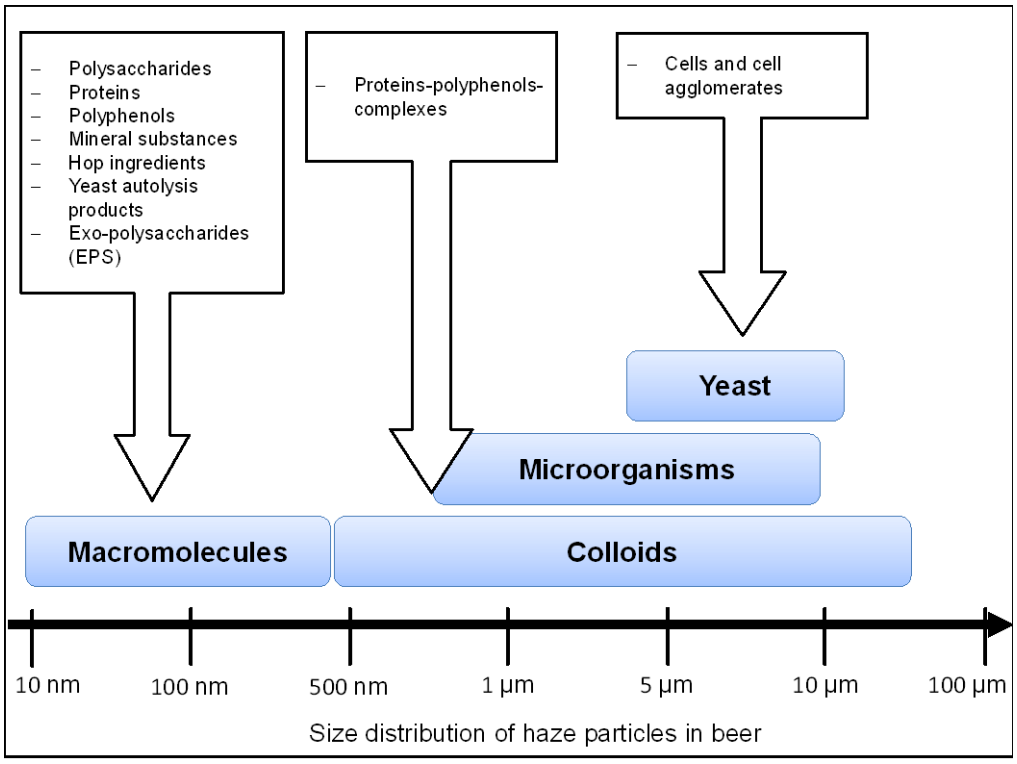

Figure 2-6: Filtration-inhibiting substances in beer in dependence on their particle size distribution [6,41,52,53]. These substances can be distinguished by macromolecules with an origin in raw materials like water, malt or hops as well as microorganisms. Furthermore, colloids resulting from protein-polyphenol complexes can be found in unfiltered beer. Finally, microorganism and yeast cells can also have an impact on the filterability of beer.

Proteins or total nitrogen content with a main source in malt and hops range regularly between 180 and 1950 mg/l in beer, whereas high molar mass fractions determined as $MgSO_4$-precipitable nitrogen have a share of 35–500 mg/l [54]. Proteins and polypeptides can have a molar mass between 5.0×10^3 and 1.0×10^5 g/mol in beer [55], which can be divided according to their molar mass into three groups: high (> 4.0×10^4 g/mol), medium (1.5×10^4–4.0×10^4 g/mol) and low (< 1.5×10^4 g/mol) molar mass fractions [56]. Low and medium molar mass fractions are important for foam stability [57,58]. Furthermore, proteinaceous substances are the main source of all turbidity in beer with a share of 75% [59,60]. Proline- and glutamic acid-rich proteins and polypeptides could be identified as the main reason, having a molar mass between 1.0×10^4 and 4.0×10^4 g/mol [56,59,61].

Polyphenols originating from malt (70–80%) and hops (20–30%) range in beer in a concentration between 40 and 400 mg/l [52,62]. Due to a high complexity of this group in dependence on polymerization degree (monomeric: <1.0×10^4 g/mol or polymeric: >1.0×10^4 g/mol) and thus molar mass range, polyphenols can be distinguished into flavanols, flavonols, flavonoids, proanthocyanodins, anthocyanogenes, tannoids and tannins [63,64]. Due to their chemical composition, polyphenols can react with proteins, resulting in the formation of haze particles. A ratio of haze-active to haze-forming polyphenols in beer of 40:1 has been found [65]. Resulting colloid particles can have sizes of 0.5–50 µm in wort and beer (see Figure 2-6) [52,53].

Polysaccharides are polymeric carbohydrates built from monosaccharides or monosaccharide derivatives linked by glycosidic bonds with a main source in malt [54,66]. Differentiation between α-, β-glucans and arabinoxylans can be made in beer. Kreisz [6] questioned the presence of extracellular polysaccharides from various microorganisms in beer. **α-Glucans or dextrins** can occur in beer as α-1,4-linked glucose units with a helical structure known as amylose and α-1,4/1,6-branched glucose units known as amylopectin originating from malt or glycogen derived from *Saccharomyces* yeast metabolism [6]. In beer, concentrations of 18–50 g/l with a molar mass range of 2.0×10^3–2.5×10^4 g/mol have been found [54]. Characterization of dextrins in the brewing industry is mostly performed using photometrical iodine values. Ranges in beer are determined between ΔE=0.02 and 1.60 [54]. Furthermore, β-linked

glucose units originating in barley or wheat with β-1,3;1,4-glycosidic linear linkages or in yeast cell walls with β-1,3;1,6-glycosidic branched bonds are known as **β-glucans**. These polysaccharides contain up to 70% β-1,4-glycosidic bonds that are interrupted by at least 30% β-1,3-glycosidic bonds in barley, which results in a linear molecule with a kink at β-1,3-linkages [67-69]. Based on their solubility, the non-extractable hemicelluloses and soluble gum can be differentiated in malt. While malting and mashing, non-water-soluble β-glucans are released from cereals like barley by glucan degrading enzymes, resulting in a reduction of molar mass [68,70]. Thus, molar masses between 2.0×10^3 and 40.0×10^6 g/mol have been detected in beer [69]. The amount of total β-glucan is described between 10 and 750 mg/l [54], whereas concentrations of up to 1100 mg/l have been detected in beer [71,72]. Furthermore, β-glucans are known to increase the turbidity and viscosity of beer due to their ability to form agglomerates known as β-glucan gels [73,74]. Clasen et al. [67] demonstrated that especially high molar mass β-glucans (>1.0×10^5 g/mol) interact via hydrogen bonds and form gels. This agglomeration can be further enhanced by low pH values, low sugar concentrations, high ethanol content as well as the action of shear forces [73]. In addition to cereal β-glucans, yeast β-glucan can be detected in beer, originating from yeast cell walls of *Saccharomyces* yeast strains. These polysaccharides have molar masses between 2.0×10^3 and 3.0×10^5 g/mol and are not able to form gels because of their branched structure [6,75]. Another β-glycosidic bound polysaccharide of the cereal cell wall is **arabinoxylan**. This polymer consists of a backbone of xylopyranosyl residues linked by β-1,4-glycosidic bonds and β-d-xylopyranosyl residues substituted at O-2/O-3 or O-2 and O-3 with a varying amount of α-L-arabinose residue. These arabinose residues are linked with β-d-xylopyranosyl at O-3 and can be substituted with ferulic acid at O-5 [76]. 210–500 mg/l arabinoxylans have been determined in lager beer [54]. A molar mass distribution in beer could not be found in literature. In addition to proteins, polyphenols or polysaccharides, **melanoidins** as well as **mineral substances** (e.g. calcium, magnesium or iron) are known to have an impact on the turbidity and filterability of beer [6,77,78].

Besides ingredients of raw materials, **microorganisms** can occur in beer due to controlled dosage or spoilage. *Saccharomyces cerevisiae* and *Saccharomyces pastorianus* spp. **yeast cells** are used for the fermentation of sugars into ethanol and carbon dioxide. Furthermore, several **autolysis and metabolism products** like

glycogen, mannan and a broad range of aroma substances can be found in beer [6]. Most aroma-active esters in beer are formed by intracellular processes catalysed by an acyltransferase or “ester synthase” during fermentation. The required energy for the reaction is provided by the thioester linkage of the acyl-coenzyme A cosubstrate, most abundant occurring as Acyl-CoA [79]. The main volatile substances that form during *Saccharomyces* spp. yeast fermentation are acetate esters of ethanol or higher alcohols (where the acid group is acetate and the alcohol group is ethanol or higher alcohol) like ethyl acetate (solvent-like aroma) or isoamyl acetate (banana aroma) as well as ethyl esters of medium chain fatty acids (MCFA; where the alcohol group is ethanol and the acid group is MCFA) like ethyl hexanoate (aniseed, apple-like aroma) or ethyl octanoate (sour apple aroma) [79-81]. Because of their lipid solubility, ethyl esters can diffuse through the cell membrane into the fermentation medium. This transfer decreases with increasing chain length of MCFA (ethyl hexanoate: 100%, ethyl octanoate: 54–68%, ethyl decanoate: 8–17%) [80,82]. In contrast, the excretion of acetate esters is rapid and complete. Variables for ester production are used yeast strain, composition of fermentation medium and fermentation conditions [80]. A high impact on volatile production was described during high-gravity brewing, with stronger oxygenation of wort, composition in unsaturated fatty acids as well as amino acid in wort [80,83,84]. For this reason, large variations in the beer aroma are possible, and minor changes in beer flavour composition could have a great impact on final beer aroma. During DE and membrane filtration trials, decreases in volatile composition could be demonstrated depending on chemical composition [85,86]. However, filtration-inhibiting effects have not yet been observed.

2.4 Thesis outline

The previous chapters pointed out that beer filtration is not only influenced by the applied filtration operations but also by the composition of the unfiltered beer. Different effects on filter performance can be determined as a function of filtration type as well as kind and composition of filtration-inhibitory substances. Investigations showed that the protein content of barley and malt had no correlation to filterability of beer. Rather, the proportion of proteins that is present after fermentation and maturation in beer apparent as haze must be considered [87]. Haze-active proteins, mainly derived from hordeins rich in prolamine, primarily influenced filter performance due to interactions

with polyphenols [56]. The impact of proteins on membrane and DE filtration are well described in literature [37,47,86,88-90]. Due to the size of occurring haze particles (compare Figure 2-6), protein-polyphenol complexes cannot enter membrane pores, resulting in a cake layer formation [86,91]. During DE precoat filtration, large amounts of high molar mass nitrogen resulted in a faster increase in pressure [88]. Moreover, it can be assumed that protein-polyphenol complexes are deposited in filter cake or precipitated by adsorption on the filter aid [92,93]. Especially the addition of hot break, with its high amount of proteins and polyphenols (65–75%), resulted in a decrease in filter performance [94-97]. A direct impact of polyphenols could be found neither in DE precoat nor in membrane filtration [86,88]. Besides colloid complexes, macromolecules can affect filtration performance (see Figure 2-6). Quantitatively, α-glucans are the largest group of polysaccharides in beer [98]. High molar mass fractions may arise due to incomplete amylolysis and result in turbidity and filtration problems. Narziss [99] determined that contents above 200 mg/l could have a negative impact in DE filtration. In particular, the presence of degradation products of amylopectin influenced cake filtration [88,93,100]. Comparable effects were found in membrane microfiltration [101-103]. Nevertheless, different authors could show a low impact of α-glucans in well saccharified beer [104,105]. Regarding filtration-inhibiting substance groups in beer, a large effect of viscosity-increasing ingredients could be shown. Especially cell wall substances of malt are known to increase beer viscosity and thus may influence beer filterability. Differentiation between arabinoxylans and β-glucans must be made because of its molecular structural differences. Negative effects on membrane filtration were shown with the dosage of arabinoxylan standards to beer [105,106]. Furthermore, Narziss et al. [104] described a slight influence of arabinoxylan on filterability. Clogging mechanisms on filter membranes were not described by the authors [104-106]. In contrast, an impact of this linear macromolecule in DE filtration could not be found in literature [107].

The largest number of investigations were found on the impact of β-glucans on filter performance during DE and membrane filtration [68,99,104,105,108-110]. According to Annemüller [111], nearly 60% of DE filter performance declines originate in the β-glucan composition of beer. In particular, several publications showed the influence of high molar mass barley β-glucan (> 1.0×10^5 g/mol) on the filter performance [110,106]. Although high molar mass β-glucans were also blamed for the clogging of

the DE precoat filter, no detailed analytical proof of this hypothesis could be found in literature. Despite the dominant role of cereal β-glucans in beer filtration, partially contradictory statements were found in literature [18,108]. Nevertheless, β-glucans definitely increase beer viscosity, which causes a proportionately higher pressure increase during filtration [73]. In this case it was assumed that β-glucan gels have a negative influence on both the filtration performance of membrane and DE precoat filtration. An imaging examination for the identification of clogging mechanisms of these polysaccharides in beer membrane and DE filtration was not performed. Furthermore, other scientists have hypothesized that the impact of cereal β-glucans on beer filterability is covered by higher concentrations of further beer ingredients like proteins [112].

Similarly, the impact of yeast β-glucans on beer filtration could not be found in literature. However, evidence on the effect of β-glucans derived from yeast cell walls on the filter performance was found in literature [6,113]. Due to cell lysis, not only yeast polysaccharides but also aroma substances can be transferred to fermentation medium. Various authors showed that acetate esters had only a low decrease during DE precoat and membrane filtration [85,86]. In contrast, it was shown that free fatty acids and MCFA ethyl esters had a higher decrease during these filtration processes [85,114]. A connection between these reductions and other beer ingredients could not be found in these publications. The addition of cell lysate, however, had a large negative impact on filter performance [6].

Controversial discussion about β-glucans resulted in the motivation to investigate the influence of these biopolymers on beer filterability in membrane and DE filtration. Due to the findings regarding beer filterability presented in the previous chapters, the following working hypotheses will be investigated in this dissertation:

- *The examination of filtration-inhibiting β-glucan molar masses observe differences in the filter performance of DE precoat and membrane filtration.*
- *Due to the branched structure of the yeast β-glucans, these polymers have a stronger tendency on filter clogging than the unbranched coiled barley β-glucans.*
- *The reduction of MCFA ethyl ester during filtration processes not only influences beer flavour but also filter performance.*

- *The interaction between β-glucans and MCFA ethyl esters results in an agglomeration of polysaccharides and a consequent decrease in filterability.*

The investigation of these hypotheses is important due to ever-increasing cost pressures and a higher degree of automation and an associated change to membrane filtration processes in the brewing industry. Because of this less variable filtration type regarding the membrane separation layer, higher demands on the beer to be filtered must be made. The comparative identification of filtration-inhibiting substances in both membrane and DE precoat filtration represents the first step towards process optimization. Besides a connection between standard analysis in unfiltered beer and filterability, an examination of the impact of concentration, the molecular structure due to different glycosidic bonds and the impact of molar masses on filtration performance should provide more knowledge about the type of filtration-inhibiting polysaccharides. In addition, the locally-resolved image analysis of filter membranes using confocal laser scanning microscopy should provide more detailed information on clogging processes influencing beer membrane filtration. The required results thus aim to optimize the beer filtration process as well as beer product quality for longer haze and flavour stability.

3 Results (Thesis publications)

3.1 Summary of results

The thesis publications are summarized in this chapter, followed by full copies of the papers.

Part 1 Page 29 Chapter 3.2	**Critical review of the methods of β-glucan analysis and its significance in the beer filtration process**

β-Glucans are polymers containing β-glycosidic linkages that occur in beer as degradation products of yeast and cereal cell walls. These polysaccharides are known to have a technological influence on the filtration performance because of their functional properties as viscous, gel-forming hydrocolloids. Because current quantification methods are based on various chemical and physical properties of these polymers, comparisons between methods are limited. Significant results concerning diatomaceous earth filter performance were achieved analysing the gel content using fluorometric methods. Furthermore, viscosity measurements yielded a good correlation with DE filtration. Informative results for membrane filtration could be obtained analysing high molar mass fractions (>9×10^5 g/mol). In addition to the cereal β-glucans, evidence of a large negative impact of yeast β-glucans could be found. Although β-glucan molecules affect both DE and membrane filtration, molar mass fractions involved and their physical properties differ, as demonstrated using the measurement methods described.

Part 2 Page 41 Chapter 3.3	**Interactions between dissolved β-glucans and medium-chain fatty acid ethyl esters in model beer solution and their impact on filterability**

As shown in previous studies, not only the concentration of the dissolved beer ingredients but also their molar mass could influence the filterability. Thereby polysaccharides of malt, especially β-glucan, are reported to have the greatest impact on filter performance. In the present study, the effects of barley (1,3;1,4) and yeast (1,3;1,6) β-glucan combined with aroma-relevant substances of beer were studied in DE and membrane filtration (polyethersulphone, 0.45 µm) using ethanolic (4% w/w) model solutions. An increasing β-glucan concentration was found to have a negative impact on both applied filter types. A concentration increase of 300 mg/l barley β-glucan decreased the filtrate flux by more than 40% during membrane filtration. In contrast, pure medium chain fatty acid (MCFA) ethyl esters had no effect on the filterability. Mixed with 1,3;1,4-β-glucan the filtrate flux decreased from 400 kg/(h×m^2) to less than 250 kg/(h×m^2). A decrease of MCFA ethyl ester ethyl dodecanoate of up to 90% was measured in the filtrate. In comparison to barley β-glucan, an equivalent concentration of yeast β-glucan caused a flux decrease of more than 95% during membrane filtration. In summary synergistic effects on filterability with polysaccharides and fermentation byproducts could be shown.

Part 3 Page 49 Chapter 3.4	**Impact of flavouring substances on the aggregation behaviour of dissolved barley β-glucans in a model beer**

Previous studies have shown that β-glucans in combination with aroma substances from yeast fermentation influenced the filtration performance of DE and membrane filtration. The impact of the beer volatiles dodecanoic acid, octyl butanoate, ethyl decanoate and decyl acetate on molar mass and radii of barley β-glucan was therefore investigated in ethanolic (4% w/w) model solution. After the addition of 100 mg/l ethyl decanoate and decyl acetate to the β-glucan solution a wider-ranging molar mass distribution could be observed by means of asymmetric field-flow fractionation. Due to agglomeration, average molar mass of β-glucan standard (M_W = 6.8×10^6 g/mol) increased by 2×10^6 g/mol ($P<0.05$) in solution containing decyl acetate. Furthermore, a significant growth ($P<0.05$) from 86 to 102 nm in gyration radius was measured. The obtained results elucidate the importance of fatty acid derived flavouring substance composition in beer regarding the aggregation behaviour of β-glucan. This agglomeration of β-glucans has a significant influence on the filtration of DE but above all membrane filtration.

Part 4 Page 57 Chapter 3.5	**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 in particular on crossflow membrane filtration were 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 a large 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 the 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 by up to 40%. In addition, the content of ethyl octanoate (30%) and ethyl decanoate (40–60%) dropped during filtration in all samples. Observed results allow specific conclusions regarding the filtration performance of beer in crossflow membrane filtration.

3.2 Critical review of the methods of β-glucan analysis and its significance in the beer filtration process

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REVIEW PAPER

Critical review of the methods of β-glucan analysis and its significance in the beer filtration process

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Abstract β-Glucans are polymers containing β-glycosidic linkages that occur in beer as degradation products of yeast and cereal cell walls. These polysaccharides are known to have a technological influence on the filter performance time because of their functional properties as viscous, gel-forming hydrocolloids. Despite knowledge of the impact of β-glucans on filterability gained using model solutions, these results can only rarely be transferred to the brewing process. Because current quantification methods are based on various chemical and physical properties of these polymers, comparisons between methods are limited. Significant results concerning kieselguhr filtration, particularly the gel content, were assayed using fluorometric methods. Furthermore, viscosity measurements yielded a good correlation with kieselguhr filtration. Informative results for membrane filtration could be obtained using chromatographic separation of the sample and detection of high molecular weight fractions (>90 kDa) using enzymatic degradation. Although β-glucan molecules affect both kieselguhr and membrane filtration, the molecular fractions involved and their physical properties differ, as demonstrated using the measurement methods described.

Keywords Beer and brewing process · Yeast · Barley · Polysaccharides · Hydrocolloids · Gels

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Introduction

Solid–liquid separation techniques are used in many industries. Filtration is used as a process step not only during the cleaning of waste water and the production of drinking water but also in the production of food [1]. Filtration procedures are often used to enhance the quality of the product, particularly in the production of beverages. Solids causing turbidity are removed from the liquid to fulfil the customers' demands for a clear, pure beverage. In addition, microorganisms are removed during filtration, resulting in an increased shelf life [2]. In the wide field of beverages, beer is considered to be more complex with respect to filtration. Procedurally, beer filtration is influenced by the dissolved ingredients of malt, hops, water and yeast, as well as their processing, e.g. wort treatment, fermentation and storage technology [2]. Despite improved technological procedures, filtration problems associated with lower filter life, and therefore a higher expenditure and costs, may still occur.

In addition to proteins and polyphenols, polysaccharides are a major component in beer. These polysaccharides are divided into starch degradation products and cell wall components such as β-glucan and arabinoxylan [3]. β-Glucans are frequently examined when considering the degrading effects of beers on the filtration processes. Although these polysaccharides occur in beer in a ratio of only 1:200 compared with α-glucans, they can have a strong impact on filtration [4]. In addition to their rheological properties, such as their ability to form gels, their molecular size, structure and origin are also of interest for further investigations. The β-glucans in beer can originate from two different sources. The water-soluble β-(1,3;1,4)-linked glucans have their origin in cereals such as barley (Fig. 1) [5]. It has been shown that the solubility, molecular weight and concentration of

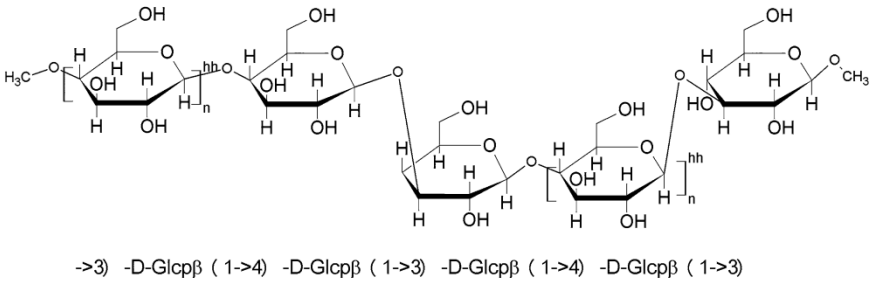

Fig. 1 Linear (1,3;1,4)-β-glucan from the cell walls of cereals, modified according to [97]

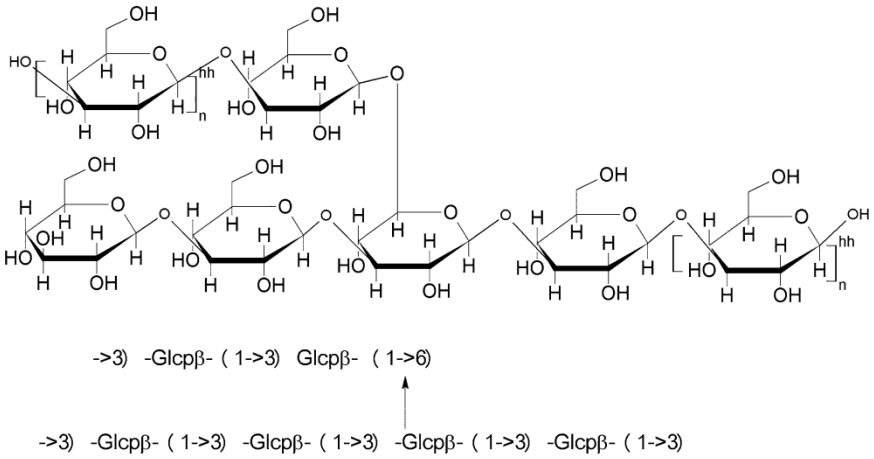

Fig. 2 Branched (1,3;1,6)-β-glucan from the cell wall of yeast, modified according to [97]

these β-glucans can be influenced during malting, primarily due to an extended germination time [6]. Depending on the mashing intensity and temperature, these polymers can enter the beer by enzymatic release and thermal hydrolysis [7]. The content of β-1,4- and β-1,3-linkages has been described as 70 and 30 %, respectively, which results from a non-branched linear homopolysaccharide [8]. Because of these cellotriose and tetraose sequences, association with other molecules via hydrogen bonding results in the formation of gels [9, 10]. The apparent concentration of β-glucans in beer varies with changes in process steps, as well as the methods used for quantification. According to Jacob and Welten [11, 12], the high molecular weight fraction (>10 kDa) occurs in a concentration range between 100 and 300 mg/L. The second source of β-glucans in beer is the β-(1,3;1,6)-branched yeast polymer, which has structure stabilising properties, from the cell wall of *Saccharomyces* species (Fig. 2) [13]. Because of its branched structure, yeast β-glucan is not known to form strong gels [9]. The complex structures, different origins and properties of these β-glucans have led the brewing industry to use various measurement methods to determine their amounts in beer samples. The use of these different techniques, each with different isolation and determination methods, leads to highly divergent and non-comparable results [14]. Utilising natural enzyme activities to determine the concentrations of beer ingredients is crucial, particularly in the context of process handling.

This review discusses the fundamental relationship between methods of β-glucan analysis and their information value, focusing on the use of beer kieselguhr and membrane filtration. Furthermore, possibilities for the

differentiation and quantification of yeast and cereal β-glucans in beer should be elaborated because no standard methods are available for the quantification of yeast β-glucans in the brewing industry.

Processes of beer filtration

Classical beer filtration is performed as a precoat filtration using diatomaceous earth (kieselguhr) as a filter aid [15]. Diatomaceous earth consists of the silica shells of fossilised diatoms. In addition to silicon dioxide, natural diatomaceous earth consists of organic additives and inorganic constituents such as calcium, iron, aluminium, magnesium, arsenic and potassium [16]. Depending on the extent of purification, natural-calcined and flux-calcined diatomaceous earth can be distinguished. Because of the wide variety of pores, channels and cracks on its surface, good filtering effects could be observed. Due to these surface properties, the particles also have a large adsorption capacity [17]. In addition to the shape and large internal surface area, the damage caused during production of the kieselguhr is also important. This affects the development and thickness of the cake formation [18, 19]. Precoat filtration with kieselguhr is usually performed in a dead-end manner. During filtration, a filter cake is deposited on a supporting sieve layer and subjected to several changes in beer filtration. In addition to the penetration of trub particles into the pores of the cake, deposition in the depth of the layer is possible. This results in a rise in pressure due to the compressibility of the filter cake. Separation of trub particles is influenced by the flow velocity and the concentration of filtration-inhibiting substances in the fluid [20, 21]. These inhibiting substances originate in the raw materials: microorganisms and yeast [13]. Table 1 gives an overview of the literature relating to β-glucans and kieselguhr filtration. A wide range of different β-glucan determination methods can be seen. Furthermore, the different substance groups studied in this context are described.

Another newer filtration method in the brewing industry is the membrane filtration, which is primarily performed using the cross-flow technique. In this type of filtration, both surface and depth filtration effects can occur [22]. The use of filter aids such as kieselguhr or perlite is not necessary. In addition to the beer parameters, the transmembrane pressure difference and the flux are important process parameters [23]. Generally, a distinction can be made between external fouling, which is an accumulation of rejected particles on the top surface, and internal fouling, which is a deposition or adsorption of small particles and macromolecules at the pore entrance or within the internal pore structure [24]. In detail, three phenomena can be differentiated: pore blocking (complete blocking of the membrane pores), direct adsorption (standard blocking) and boundary layer resistance (cake formation) [24–26]. Macrosolutes and colloids could be responsible for pore plugging because of their high tendency to interact with the membrane material [27]. Substantial amounts of research have been done to determine the reasons for the increase in pressure during filtration. An overview of the different studies is given in Table 2. In addition to the wide examination of different beer ingredients, a variety of β-glucan measurement methods have been applied, which have resulted in different information with respect to this polysaccharide.

Detection of β-glucans in beer

Various methods for the detection of cereal β-glucans in beer have been developed over the years. These methods are based on different properties of β-glucan. Yeast β-glucan can also be included in beer, but it cannot be detected with the available methods of analysis [11, 12]. In addition to direct quantitative determination, such as precipitation, enzymatic or staining methods, the ability of β-glucan to increase the viscosity of aqueous solutions can be used as an indirect measurement method for determining the amount of β-glucan [4, 28–32].

Table 3 describes the four fundamental measuring principles used in β-glucan determination and the molecular sizes that can be detected. The applied methods detect β-glucans with a wide diversity of molecular sizes, resulting in different information regarding the types of beer filtration. Depending on the application, conflicting predictions for the process of beer filtration can be obtained. In terms of good process control, an early knowledge of the composition of beer is essential. Methods must be used that provide consistent information for the particular filtration.

Methods used for β-glucan detection in kieselguhr filtration

Several methods for the detection of β-glucans that are used to predict kieselguhr beer filtration are described in the literature. During filtration trials, investigations using viscosity measurement were made to predict filterability. Schimpf et al. [33] showed that no correlation could be found between wort viscosity and beer filterability, while beer viscosity could be correlated with kieselguhr filtration tests. This emphasises the effects of fermentation and storage on beer composition and filterability. Furthermore, the presence of solvents, such as ethanol, or different oligosaccharides affects the behaviour of β-glucan in solution [34–36]. Thus, the association between β-glucan molecules is reinforced by fermentation and the influence of ethanol, whereas the presence of oligosaccharides such as maltose

Table 1 Literature dealing with kieselguhr beer filtration and β-glucans stating the methods of measurement and other investigated beer ingredients

Measurement method for β-glucan	Studied ingredients and characteristics	Source
Freeze-dried β-glucan gel	Yeast, hot break, cold break, β-glucan gel	[56]
Enzymatic measurement of glucose (degradation using acid hydrolysis)	β-Glucan	[4]
Indirect measurement using the degradation of β-1,3-glucanase, β-1,4-glucanase and hemicellulose in combination with flux increase	Viscosity, extract difference, β-glucan, 'gums', proteins, turbidity	[50]
Enzymatic method, total β-glucan content, fluorometric measurement using FIA	β-Glucan, viscosity	[51]
Precipitation with 30 % $(NH_4)_2SO_2$ and degradation using phenolic sulphuric acid with measurement of the resulting glucose concentration	β-Glucan, turbidity, viscosity, total nitrogen, unresolved nitrogen, organic substances, proteins involved in filter cake, glucose, ether-soluble substances, beer age, original gravity	[33]
	β-Glucan, 'gums', viscosity of the mash, malt solution, malt quality, malting temperature, viscosity of the wort, pH value, glycerol, $MgSO_4$-precipitable nitrogen, coagulated nitrogen, total nitrogen, turbidity	[86]
Precipitation with $(NH_4)_2SO_2$ and degradation using H_2SO_4 with measurement of the resulting glucose concentration	β-Glucan, gums, turbidity, viscosity, total nitrogen, high molecular weight nitrogen, α-glucan, dextrin, polyphenols	[42]
Precipitation with 30 % $(NH_4)_2SO_2$ and degradation using phenolic sulphuric acid with measurement of the resulting saccharide concentration	β-Glucan, proteins, trub content, polysaccharides of the trub, trub ash content	[43]
No named method	Yeast, hop resins, bacteria, proteins, α-/β-glucan, arabinoxylans, melanoidins, mineral substances, polyphenols	[87]
Fluorometric assay Analytica EBC 3.11.2	Yeast, total nitrogen content, $MgSO_4^-$-precipitable nitrogen, α-/β-glucan, total polyphenols, anthocyanogenes	[60]
Fluorometric measurement using FIA, measurement of β-glucan-gel	β-glucan, β-glucan gel	[58]
Fluorometric measurement using FIA, gel determination according to Wagner [51], dynamic light scattering	Viscosity, β-glucan, β-glucan gel, arabinoxylans	[14]
MEBAK II 2.5.2 (fluorometric assay) Residue in the kieselguhr cake using NaOH hydrolysis combined with fluorometric measurement	β-glucan, mannan, dextrane, xanthan, glycogen, yeast	[13]
Fluorometric measurement using FIA gel determination according to Wagner [88]	Nitrogen, polyphenols, β-glucan, β-glucan gel, viscosity	[57]

Table 2 Literature dealing with membrane beer filtration and β-glucans stating the methods of measurement and other investigated beer ingredients

Matrix	Measurement method for β-glucan	Membrane quality	Studied ingredients and characteristics	Source
Beer	–	Ultrafiltration membranes (cut-off 10,000–100,000 Da)	Colloidal stability, extract, attenuation, colour, bitterness, total polyphenols, proanthocyanodins, viscosity	[62]
Phosphate-buffered saline model solution (pH 7.4)	–	Cellulose acetate, 0.07 µm	Yeast, protein (BSA; 60 kDa)	[89]
Beer	–	Tubular ceramic media, 0.10, 0.45, 0.80 and 1.40 µm	Yeast cells, density, viscosity, pH value, dry matter, haze, proteins, polyphenols, particle distribution	[25]
Yeast cells in deionised water or wort	–	Polysulphone, 0.45 µm	Yeast cells (*Saccharomyces cerevisiae*)	[90]
Beer	Total carbohydrate assay using the anthrone reagent based upon the EBC standard	Mixed esters of cellulose, 0.45 µm	Carbohydrates, polyphenols, protein, yeast	[91]
Beer	No named method	Polypropylene (0.2–0.4 µm), polysulphone (0.4 µm), polyethersulphone (0.3 µm), Al_2O_3 ceramic (15–20 µm), ZrO_2 ceramic	Original gravity, ethanol, spec. weight, extract, degree of fermentation, pH value, colour, bitter units, polyphenols, anthocyanogenes, soluble nitrogen, viscosity, foam stability, β-glucan, shelf life	[63]
Beer	No named method	Nylon, 0.45 µm	Anionic ions, polyphenols, β-glucan, β-glucan-gel, α-amino nitrogen, coagulable nitrogen, total nitrogen	[92]
Beer	Dosage of barley β-glucan	Hydrophobic polytetrafluoro-ethylene and hydrophilic surface-modified polyethersulphone, 0.45 µm	β-Glucan, yeast, polyphenol-protein-carbohydrate haze	[93]
Beer, wort	Lichenase degradation and dosage of barley β-glucan standards (31, 137, 250, 327, 443 kDa)	'Acetateplus' plan membrane, 0.45 µm	Turbidity, shearing, pH value, temperature	[94]
Beer	β-Glucan standards	Cellulose acetate, 0.45 µm	β-Glucan, pH value, ethanol, shearing, storage time	[66]
Wort		Polytetrafluoro-ethylene, 0.45 µm	β-Glucan, proteins	[95]
Beer, wort	Double-enzyme method of McCleary and Glennie-Holmes in combination with gel chromatography	Polyethersulphone, 0.2 µm	β-Glucan, α-glucan, arabinoxylan, viscosity	[52]
Beer	Indirect measurement using the degradation of fungal β-glucanase in combination with flux increase	Tubular ceramic membranes (ceramic monolith), 0.5 µm	Deposited minerals, indirect analysis of starch, β-glucan, pullulan, proteins and cellulose to enzymatic degradation	[64]
Beer	Mixed linkages β-glucan assay kit	Polyamide, 0.45 µm	Proteins, polyphenols, β-glucan, arabinoxylan, Kolbach index, viscosity	[53]
Ethanolic (6 % (v/v)) acidic model solution (pH 4.2)	Three enzymatic degraded barley β-glucans	Nylon, 0.45 µm	β-Glucan, arabinoxylans, dextrin, BSA, viscosity	[65]

Table 2 continued

Matrix	Measurement method for β-glucan	Membrane quality	Studied ingredients and characteristics	Source
Beer	Enzymatic kit by Megazyme International Ltd., enzymatic degradation using cellulose (C2605, from *Aspergillus* spp.)	Polyethersulphone, 0.45 µm	β-Glucan, FAN, fermentable sugar, photon correlation spectroscopy to measure permeate quality, by analysing the light scattered by the colloidal particles	[96]
Beer	$(NH_4)_2SO_4$ precipitation	Cellulose acetate, 0.3 µm	β-Glucan, β-glucan-gel, 'gums', viscosity, α-glucan, protein–polyphenol complexes, trub and solids content, pH value	[49]
Beer	Fluorometric measurement using FIA, gel determination according Wagner [51], to dynamic light scattering	Cellulose acetate, 0.2 µm	Viscosity, β-glucan, β-glucan gel, arabinoxylans	[14]

Table 3 Determination methods for β-glucan in cereals, wort and beer stating measurement principle and detection limits

Method	Principle	Detectable molecular sizes	Source
Viscometric measurement	Determining the toughness of a solution using rolling ball, capillary and rotational viscometer [12]	Total β-glucan [31]	EBC (Method 4.18, 9.38), MEBAK (Method 2.25), ASBC (Method Beer 32 Viscosity)
Precipitation	Precipitation of β-glucan using reagents and measurement of the hydrolysed glucose monomers [41, 45]	>12 kDa [4], ~300 kDa [33]	Analysenmethoden für die Brau- und Malzindustrie. (Method 2.1.13) [41] (outdated method), [46]
Enzymatic degradation	Enzymatic breakdown of β-glucan and measurement of the hydrolysed glucose monomers [45]	Total β-glucan [28]	AACC (Method 32-23.01), EBC (Methods 3.11.1, 4.16.1 and 8.11.1), MEBAK 2.5.1
Staining	Calcofluor white staining and measurement of fluorescence intensity (adsorption 360 nm, emission 425 nm) [11]	>10 kDa [46], ~50 kDa [30]	EBC (Method 3.10.2, 4.16.2, 8.13.2), ASBC (Method wort 18.A), MEBAK (Method beer, wort, beer-based beverages 2.5.2)
	Congo red staining and measurement of the adsorption (550 nm) [30]	~250 kDa [30]	MEBAK 2.5.4

~ Average molecular size

counteracts the degree of association [34, 37]. In addition to ethanol, precipitation products of yeast ingredients and cell decomposition can influence this behaviour and, thus, the viscosity and filterability [13, 38]. Using dynamic viscosity measurements for the detection of intrinsic viscosity, a high correlation ($R^2 = 0.781$) with filterability could be observed; suspensions with an intrinsic viscosity greater 1000 mL/g resulted in massive filtration difficulties. The author ascribed these modifications to changes during fermentation, as well as the nature and characteristics of yeast cells (e.g. death proportion) [14]. This intrinsic viscosity specifies the required volume of 1 g of dissolved polymer in the investigated solvent. Considering influencing factors on viscosity measurement, intrinsic viscosity provides more detailed information, such as chain stiffness and molecular dimension, about the studied polysaccharides [39, 40]. Nevertheless, viscosity measurements describe only the overall behaviour of solutions; specific evidence concerning the presence of β-glucans in polymer mixtures is not obtained.

Over the years, new methods for the detection of β-glucans have been discovered. These methods are based on the extraction and precipitation of β-glucans. Quantitative determination could be accomplished using a gravimetric method [41] or hydrolysis of the precipitated polymers to its monomers and a measurement of the amount glucose [4, 33, 42]. Schimpf et al. [33] showed significant correlations among the β-glucan content of wort, its kieselguhr filterability and the content of glucose residue in the filter cake ($r = -0.67$). The β-glucan content was determined using precipitation with 30 % ammonium sulphate and degradation to glucose with phenolic sulphuric acid hydrolysis. Other authors have also observed the negative effect of increasing β-glucan concentration on kieselguhr filterability [42, 43]. The specificity of these methods has been questioned by several authors [8, 44]. During precipitation, both β-linked polymers and non-β-glucan polysaccharides may be precipitated, whereas the β-glucan content could be overestimated because of the presence of α-glucans [44–46]. Furthermore, precipitating agents support the association of polymers via hydrogen bonding by removing the hydration shell. This results in incorrect evidence during the course of β-glucan molecular size determination [47, 48]. A further disadvantage is the missing single analytical principle for the detection of β-glucans in beer, wort, malt and barley [44]. Due to changes in the polymer structure during fermentation and interactions with other oligosaccharides, measurement of the β-glucan content of wort is not practicable [14]. Additionally, the applied methods are not able to differentiate between β-glucan gel and sol [49]. For a more accurate determination of β-glucan, enzymes that specifically degrade these polymers were examined.

Similar results were achieved concerning kieselguhr filtration. An improvement in filter performance could be demonstrated after enzymatic degradation of structural substances [4, 50]. Further investigations showed that the determination of β-glucan concentration using the enzymatic method described by McCleary had no informational value concerning kieselguhr filtration performance [51]. The overall content of β-glucan does not provide information for beer filterability [52, 53]. Only the higher molecular weight fraction seems to affect the filterability. Furthermore, a distinction between β-glucan gel and β-glucan sol is not possible.

The determination of β-glucan gels is a benefit of the fluorometric method. To take advantage of the conversion of a gel to the sol state by thermal treatment, the samples are heated for 20 min at 80 °C. The difference in glucan concentration after and before heating corresponds to the content of β-glucan gel [54]. The basis of this method is the complex formation of the fluorochrome calcofluor (Fig. 3) during which cereal β-glucans form hydrogen bonds, van der Waals forces and ionic interactions at alkaline pH (pH 10) [55]. The complexes could be excited to fluorescence by UV light (adsorption 360 nm, emission 425 nm). The negative effect of β-glucan-gel content on kieselguhr filtration could be shown with the help of a calcofluor assay in combination with a flow injection apparatus (FIA) [56–59]. A fractionation of β-glucan confirmed the results of Wagner [51] that beer contains mainly high molecular weight β-glucans [60]. However, no correlation between β-glucan concentration, determined using the

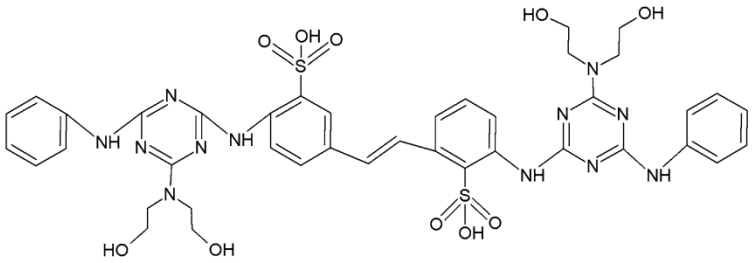

Fig. 3 Dye calcofluor (2,2′-[(E)-1,2-ethenediyl] bis[5-({4-anilino-6-[bis(2-hydroxyethyl)amino]-1,3,5-triazin-2-yl}amino)benzenesulphonate]) used for the fluorometric determination of β-glucans shown in the deprotonated state, modified according to [98]

fluorometric method, and filtration behaviour was found by the authors [51, 60]. Kreisz [13] examined the filterability of beer and wort depending on different polysaccharides. Analysing the β-glucan content of the kieselguhr filter cakes, an increasing β-glucan concentration could be observed with a decrease in filterability. For the Congo red assay, no data were presented in the context of this type of filtration.

No results could be found connecting yeast β-glucan content with kieselguhr filtration. Nevertheless, conclusions can be obtained from filtration after the addition of cold break, because yeast degradation substances can be included in these fractions. Masák et al. [43] showed that an increasing addition of cold break separated from fermented beer resulted in a declining filterability. The investigated trub contained 23 % hydrolysed polysaccharides, which were quantified using acid hydrolysis and determination of the resulting glucose by HPLC. Other author groups were also able to confirm the influence of cold break on filtration [33, 56, 61]. Kreisz [13] studied the impact of different yeast substances on filterability. Since neither mannan nor glycogen impaired filterability, the author concluded there must be a large influence of cell wall β-glucan, as cells lysed by shearing strongly degraded filterability.

In summary, kieselguhr filtration is influenced by the high molecular weight β-glucans, but smaller fractions could also affect the filterability due to interactions with other beer ingredients. Significant results were achieved with the fluorometric method, especially with respect to the determination of gel content. Using the determination of viscosity as sum parameter, the kieselguhr filterability showed best correlation. The negative effects of dead yeast cells as well as cold break on diatomaceous earth filtration could be shown [2, 13, 43, 56], but the effects of branched yeast β-glucans in beer could not be shown because the existing methods do not allow differentiation of the β-glucans.

Methods used for β-glucan detection in membrane filtration

Various beer ingredients have been shown to cause problems during membrane filtration. Cell wall components such as β-glucan and arabinoxylan were observed as the main cause. A decline in the viscosity of the permeate (filtrate) that depended on the filter pore size and membrane material used could be observed during membrane filtration experiments, while the viscosity of the concentrate increased [62, 63]. In this context, a drop in viscosity and β-glucan content with decreasing pore size could be observed [63]. A method for the detection of the β-glucans was not named by the author. Determining the intrinsic viscosity of different beer samples, a correlation ($R^2 = 0.846$) with membrane filterability could be made [14].

No correlations between ammonium sulphate-precipitable β-glucan content and filterability could be observed [49]. The authors conclude that the applied method lacks the specificity required to detect gel-building β-glucans.

Enzymatic degradation of β-glucans using bacterial and fungal β-glucanase from *Bacillus subtilis* and *Penicillium funiculosum*, as well as lichenase from *Bacillus* sp., has been shown to cause an increase in membrane filterability [64, 65]. The investigations using beer samples revealed no correlation between β-glucan content and filterability. A reason might be that the determination of total β-glucans using the enzymatic method did not allow differentiation of molecular weight or particle size, according to McCleary [53]. A more accurate method was performed by Narziss et al. [52]. They detected three classes of high molecular weight β-glucan fraction (>90, >200 and >750 kDa) using gel chromatography, as well as a total content β-glucan analysis using an enzymatic assay, and observed their effects on the resulting filtration behaviour. During membrane filtration with polyethersulphone membranes, they observed highly significant correlations between these molecular fractions of β-glucan and the filterability of the beer. The total concentration of the polymers had no correlation with filtration performance. The results are shown in Table 4. In addition to the β-glucan contents of the studied beer samples, the relationship between the content in wort and the residues in the filter has also been characterised. The concentrations of the fractionated samples showed highly significant correlations between the polysaccharide content of the wort and filterability, whereas the retained contents in the filters displayed a significant correlation. These results were confirmed by other authors [53, 66], wherein the total concentration of β-glucan provides no forecast of the filterability, although high molecular weight

Table 4 Relationship between filterability of beer and β-glucan concentration in wort and unfiltered beer and the β-glucan content held back by the filter (i.e. 'lost') [52]

	Correlation coefficients filterability versus polysaccharide concentration		
Polysaccharide Size (dalton)	Wort ($n = 40$)	Beer ($n = 73$)	Lost ($n = 73$)
β-Glucan			
Total	−0.16	−0.15	−0.35**
>90,000	−0.70***	−0.62***	−0.34**
>200,000	−0.71***	−0.59***	−0.35*
>750,000	−0.69***	−0.53***	−0.41***

* $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$

β-glucan fractions are essential for filtration behaviour. However, the enzymatic methods do not allow differentiation between high and low molecular weight fractions. A differentiated analysis of the ingredients is necessary because model solution studies showed that high molecular weight β-glucans, in particular, affected this type of filtration [53, 66].

Using a post-column calcofluor-FIA method, a combined determination of the amounts of different molecular weight fractions of β-glucans in cereal extracts could performed, which result in structural information about the samples [67]. No literature mentioning this assay in combination with filterability could be found. The β-glucan-gel content was found to have negative effects on membrane filtration [14]. A further possibility of applying a specific dye reaction is the Congo red assay. This assay is based on the specific reaction occurring between β-glucans and the dye Congo red (Fig. 4) at pH 8 [68, 69]. After incubation at room temperature for 20 min, the absorbance is measured at 550 nm and compared with that of a blank reaction mixture [30]. Reasons for this specific binding to glucose units linked by β-glycosidic bonds include electrostatic and hydrogen-bonding interactions, as well as van der Waals-type attractions of the two aromatic moieties of the dye with the corresponding hydrophobic cellulose surface [70]. Investigations of Anderson [30] showed that the Congo red reaction is optimally sensitive to β-glucans with a molecular weight of approximately 250 kDa. This suggests that this method particularly detects high molecular weight polysaccharides. No information about filterability and β-glucan content using the colorimetric Congo red assay is available. Comparing the results with the previously mentioned findings of Narziss [52], it seems most likely that the Congo red method could provide effective results for filterability.

In addition to cereal β-glucan, yeast β-glucan was also investigated during filtration processes. In wine production, yeast contact over a period of 5 days after the end of fermentation showed a decrease in filtration behaviour [71]. The authors were able to ascribe the flowrate degradation to the content of yeast β-glucan using enzymatic degradation. The product used in this application is Glucanex (Novozymes), a yeast lytic enzyme with the ability to break β-1,3 and β-1,6 bonds [72]. Similar enzyme kits are applied to the measurement of polymer solutions and to the quantification of cell wall components [73–75]. Due to the enzyme's ability to break barley as well as yeast β-glucan, the described method is not specific enough to differentiate between these two polymers in liquid mixture [76]. An improvement in filterability using a glucanase for the degradation of cell wall polysaccharides was also reported by Villettaz et al. [77]. Newer postulated enzymes exhibit higher specificities for β-1,3-glucosidic bonds [78]. An enzymatic degradation in combination with the detection of the mono- and oligosaccharide distribution could allow an indication of the concentration of yeast β-glucan in beer. Problems in the filtration of yeast beer were observed by other authors; thus, a negative impact of yeast β-glucan on beer filtration is presumable [56, 63, 79]. The microscopic identification of haze from barley and yeast using different solubility properties of the two polymers could be followed using Congo red staining. A quantification of the different polymers was not possible using this imaging technique [80]. However, differentiation among other glucans was not possible. A colorimetric determination of β-1,3-1,6-glucans has been reported by Mölleken et al. [81]. This method uses a standard calibration performed using a schizophyllan standard. The described method could only distinguish between different β-1,3-1,6-glycosidic bonds and the amount of total β-1,3 bonds but not between the content of β-1,3;-1,4-linked polymers [81]. This does not allow quantification of yeast β-glucan in the beer. An interaction between the helical polysaccharides in the cell wall of *S. cerevisiae* brewers' yeast and Congo red could also be shown [82]. A further method described in the context of yeast β-glucans is the Fungitell® assay for the measurement of β-1,3-glucans originating in *Candida* spp., *Aspergillus* spp. as well as *S. cerevisiae* [83]. The measurement technique is based on the release of activated serine proteases that cleave p-nitroaniline (pNA) from a peptide substrate. The resulting amount of pNA is measured spectrophotometrically at 405 nm [84]. A distinction between cereal and yeast β-glucan could be considered.

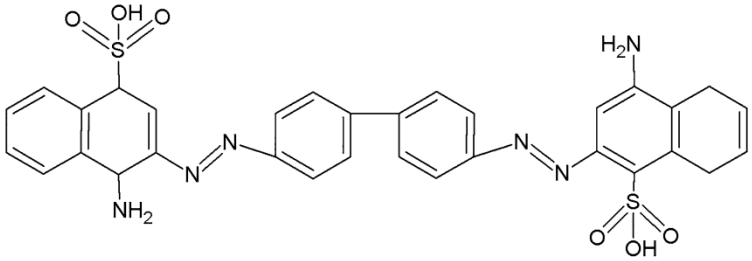

Fig. 4 Dye Congo red (3,3′-[4,4′-biphenyldiyldi(E)-2,1-diazenediyl]bis(4-amino-1-naphthalenesulphonate)) used for the detection of β-glucan in a colorimetric assay, modified according to [98]

In summary, beer filtration using the membrane method is influenced by high molecular weight β-glucans. In addition to sample fractionation using gel chromatography in combination with an enzymatic determination of the polysaccharides, the use of a Congo red assay can provide information. In addition to the cereal glucan fractions, the β-1,3-1,6-β-glucans originating in yeast have to be considered in this context. A practical method to differentiate polymers during beer filtration does not exist.

Conclusion

The effect of β-glucans on filterability seems to be a well-known and well-studied problem. Nevertheless, on closer examination, several gaps in relation to different polymers can be found. β-Glucans could be demonstrated to have a major impact of filtrations using diatomaceous earth or filtration membranes. According to the literature discussed, both the overall β-glucan content and, in particular, the amounts of higher molecular weight fractions are important for the prediction of filtration behaviour. Not all of the methods considered allow useful conclusions in relation to the filterability of beers. This can result in an incorrect interpretation of the concentrations of β-glucans, which leads to contradictory statements regarding the filterability. Because the composition of beer is very complex, viscosity measurement is a way to determine the concentration impact and the agglomeration potential of polymer mixtures. The results show that simple viscosity measurements do not provide meaningful information concerning the filterability of beers. Rather, instruments of dynamic or specific viscosity measurement must be applied. These methods yield important information for both types of filtration. Chromatographic fractionation of the sample is indispensable for a more accurate determination of filtration behaviour. However, not only the concentration and molecular weight but also the shape (gyration and hydrodynamic radius) of the polymers are of interest and should be topics of further investigations. Moreover, the aggregation behaviour and molecular association must be considered in the context of filterability. These irregular fractal aggregates can interact with other polymers, e.g. arabinoxylans, and enter the filter pores [36, 85]. The filtration effects of linear β-(1,3;1,4)-cereal glucans have been well investigated using standards, as well as with direct measurements in beer samples. Furthermore, the differentiation between cereal and yeast β-glucan is of major interest because of their specific structures and their association with filtration behaviour. The development of a method suitable for the quantification of yeast β-glucan in beer would be an important basis for the improvement of filterability.

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Compliance with Ethical Standards

Conflict of interest None.

Compliance with Ethics requirements This article does not contain any studies with human or animal subjects.

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3.3 Interactions between dissolved β-glucans and medium-chain fatty acid ethyl esters in model beer solution and their impact on filterability

Interactions Between Dissolved β-Glucans and Medium-Chain Fatty Acid Ethyl Esters in Model Beer Solution and Their Impact on Filterability

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ABSTRACT

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As shown in previous studies, not only the concentration of the dissolved beer ingredients but also their molecular size could influence their filterability. Therefore, polysaccharides of malt, especially β-glucan, are reported to have the greatest impact on filtration behavior. In the present study, the effects of barley (1,3;1,4)- and yeast (1,3;1,6)-β-glucan combined with aroma-relevant substances of beer were studied in Kieselguhr and membrane filtration (polyethersulfone, 0.45 µm) using ethanolic (4% w/w) model solutions. An increasing β-glucan concentration was found to have a negative impact on both applied filter types. The concentration increase of barley β-glucan to 300 mg/L decreased the filtrate flux more than 40% during membrane filtration. In contrast, pure medium-chain fatty acid (MCFA) ethyl esters had no effect on filterability. Mixed with (1,3;1,4)-β-glucan, the filtrate flux decreased from 400 kg/(h × m^2) to less than 250 kg/(h × m^2). A decrease of MCFA ethyl ester up to 90% was measured in the filtrate. Compared with barley β-glucan, an equivalent concentration of yeast β-glucan caused a flux decrease of more than 95% during membrane filtration. In summary, synergistic effects on filterability with polysaccharides and fermentation byproducts could be shown.

Keywords: Beer filtration, Diatomaceous earth, β-Glucan, MCFA esters

Beer filtration is a final step in beer production. The aim of filtration is to separate haze particles and microorganisms and to maintain the chemical and physical composition of beer over a long storage period (47). This is particularly important due to the long distribution chain that beer has to cover. In addition to processing time and filter service life, the turbidity at the filter outlet is crucial for the final beer quality. These process parameters are influenced by the chemical composition of the unfiltered beer (24). In particular, α- and β-glucans, arabinoxylans, protein-polyphenol complexes, melanoidins, and yeast cells are described to be substances influencing filtration (3–5,33,39,42).

The distribution of these ingredients concerning filterability was described by Annemüller and Manger (2), with approximately 60% high molecular weight β-glucan, 20% protein-tannin complexes, 15% high molecular weight α-glucan, and, finally, high yeast concentrations and microbial infections. The most influential component, β-glucan, can occur in beer because of two sources: cereals such as barley, and yeast. The linear homopolysaccharide (1,3;1,4)-β-glucan from cereals such as barley and oat have molecular weights between 1.5 × 10^2 and 2.5 × 10^3 kDa (29). According to Stewart et al. (44), a decrease in filtration performance could be measured at molecular sizes larger than 100 kDa. Additionally, the concentration of β-glucan critical for filtration is vastly dependent on molecular size of the polymers in beer (20). However, not only could β-glucan affect filterability but also its ability to form gels influences the behavior of beer during filtration (19). The tendency for spontaneous gelation rises with increasing β-glucan concentration (c = 1% w/w) independently from molecular size. Below this critical concentration, the association occurs only in the presence of high molecular weight fractions (>100 kDa) (6). Further factors which positively influence the gelation are low temperature, low sugar concentrations, mechanical stress, and high ethanol contents (25,43). Gelation can occur in cereal β-glucan because of the irregularly long sequences of (1,4)-β-linked glucopyranosyl units associated by hydrogen bonds which form junction zones between the molecules (11). Another source may be the cell wall of *Saccharomyces* yeasts. The yeast-borne (1,3;1,6)-β-linked glucans have a molecular weight of 2 × 10^1 to 3 × 10^2 kDa (24). Kreisz (24) showed that the addition of damaged yeast cells to beer has a negative impact on filterability. Effects of yeast cell wall components could also be observed in wine filtration (51). The β-glucan from *Saccharomyces* yeasts is not known to have strong gelation behavior. The molecules only build structured liquids or weak gels due to the (1,3;1,6)-β-branched, helical structure (11).

Because of the complex mixture, many research groups have tried to describe beer filtration using model solutions (10,15,26,46). Eagles et al. (10) investigated the fouling in microfiltration using a model solution containing casein, starch, ethanol, glycerol, maltose, citric acid, calcium ions, catechin, and ethyl acetate. During cross-flow filtration, they showed a decrease in flux because of the retention of polysaccharides and proteins, wherein the starch dominated this behavior. The aroma compound ethyl acetate, however, showed only a small effect on the filtration performance. The flux decline decreased in the presence of starch but not in the presence of protein. A reduction of 20% of ethyl acetate could be measured, which was regarded as an interaction with the cellulose nitrate membranes. An examination of filter performance and beer quality was conducted by Walla (49). Comparing Kieselguhr and membrane filtration (polysulfone capillaries, 0.2 µm), differences in aroma composition could be shown. In addition, filtration-inhibiting substances such as β-glucan, proteins, and polyphenols decreased during membrane filtration. These results suggest that beer filtration is affected not only by dissolved polysaccharides and proteins but also by flavor substances. In addition to acetate esters (e.g., ethyl acetate and isoamyl acetate), medium-chain fatty acid (MCFA) ethyl esters are also formed during fermentation (37). The resultant MCFA ethyl ester in alcoholic beverages carry fatty acid residues between C_6 and C_{12} (18,40). Their concentrations differ with respect to yeast fermentation performance (40,45). The filtration effects of the MCFA ethyl esters mixed with dissolved polysaccharides are investigated in this article. Furthermore, the effects of barley and yeast β-glucan during dead-end Kieselguhr and membrane filtration are studied.

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EXPERIMENTAL

Materials

To investigate the influence of the cell wall polysaccharides in beer, (1,3;1,4)-linked β-glucan from barley (middle molecular weight fraction, 262 kDa; Megazyme International Ireland) and (1,3;1,6)-linked β-glucan from yeast (Megazyme International Ireland) were used for the experiments. The studied aroma compounds included ethyl hexanoate (Fluka 21550; Fluka Analytical), ethyl octanoate (Schuchardt OC129; Merck Schuchardt OHG), ethyl decanoate (Fluka 21430; Fluka Analytical), ethyl dodecanoate (Schuchardt LA067; Merck Schuchardt OHG), and isoamyl acetate (Schuchardt ES046; Merck Schuchardt OHG). In addition, ethanol from Merck was used for a more realistic simulation of the beer composition.

For the filtrations, polyethersulfone membranes (Sartorius AG) with a pore size of 0.45 µm and area of 17.34 cm^2 were applied. The fine and coarse diatomaceous earth (permeability: 0.1/1.25 Darcy) from Begerow (Eaton Technologies GmbH) was used for the Kieselguhr precoat filtration. For each experiment, new membrane coupons as well as a new Kieselguhr were used.

Methods

Preparation of the model solutions. In order to dissolve the powdered polysaccharides, the desired amount was weighed and diluted in 200 mL of distilled water. The suspension was heated on a hot plate until boiling. The temperature was maintained for 10 min. The solution was cooled to 20°C and weighed to 2 kg with distilled water, ethanol (4% w/w), and the flavoring substances from a stock solution. This stock solution contained isoamyl acetate (1.20 g/L), ethyl hexanoate (1.55 g/L), ethyl octanoate (1.75 g/L), ethyl decanoate (1.75 g/L), and ethyl dodecanoate (1.80 g/L). The model solutions were freshly prepared before each filtration trial.

Filterability during Kieselguhr and membrane filtration. The dead-end filtration trials were performed on an automatic laboratory filter system (27,28). The automatic filter consisted of two stainless-steel vessels with cooling jackets. Pressure and temperature sensors as well as an automatic valve were connected via a controller with a computer. For data recording and control, the program Virtual Expert (Gimbo mbH) was used. In addition to temperature and pressure, the measured variables were filtrate mass and time. The obtained data were analyzed using MATLAB (MathWorks).

Because Kieselguhr filtration is the most applied method in the brewing industry, these filtration trials were performed to compare with membrane clarification. For the Kieselguhr precoat, coarse Kieselguhr at 0.58 g/L diluted in 1.7 L of distilled water was dosed into the precoat vessel. A 1-L sample with fine Kieselguhr at 0.8 g/L was filled into the sample vessel. After precoating on a 15-µm steel mesh sieve, the sample was filtered through the coarse Kieselguhr layer by switching the automatic valve.

For membrane filtration, only the sample vessel and the filter were used. Therefore, 200 mL of sample was filled into the sample vessel and flushed through the polyethersulfone (PES) membrane using CO_2 pressure. The filtration parameters were pressure at 1 bar and temperature at 5°C.

Detection of the aroma substances. For the measurement of aroma substances, solid-phase microextraction enrichment (SPME) was applied. SPME is considered to be a semiquantitative method. Because of the different polarities of the aroma substances, an SPME-holder and fiber (Stable Flex Divinylbenzol/Carboxen/PDMS, 50/30 µm, gray) from Supelco was used. Before the first use, the new fiber was conditioned for 1 h at 270°C in a helium-flushed injector of the gas chromatograph (GC). To avoid a memory effect, the fiber was heated between every GC analysis for 5 min at 250°C. The enrichment took place at 25°C (±5°C) for 30 min in a headspace glass. Thereafter, the fiber was injected for 30 s in the GC. Each sample was analyzed in duplicate.

The GC analyses were performed with a Siemens SiChromat 3 gas chromatograph (Siemens) with a Merck-Hitachi D2500 Integrator. For the detection of the aroma substances, a DB5 (J&W) (30 m by 0.25 mm, 0.25-µm film thickness) (Agilent Technologies) was used. The inert carrier gas helium was used with a volume flow of 1 mL/min (60°C) in a split ratio 1:20. The detector gases were hydrogen and air (each 2 bar). The samples were analyzed using the following temperature program: 100°C (5-min isothermal) heating at 5°C/min to 250°C. After a run time of 25 min, the analysis has been completed and the device was prepared for the next run. Prior to the next analysis, the SPME fiber was reconditioned at 250°C for 15 min (23).

RESULTS AND DISCUSSION

In order to determine the function of polysaccharides and aroma substances in membrane and Kieselguhr filtration, the filterability of different model solutions was measured. For simula-

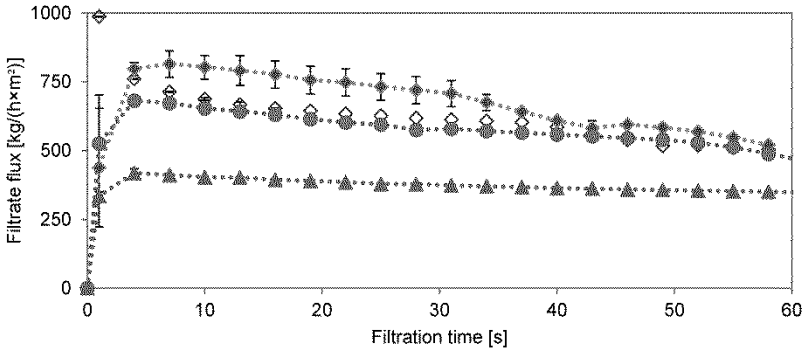

Fig. 1. Average filtrate flux (n = 3) of the microfiltration profile using polyethersulfone membranes of synthetic polysaccharide model solution containing barley β-glucan in an amount between 0 and 300 mg/L. β-Glucan concentration of the solution: ◇ = 0 mg/L, ◆ = 100 mg/L, ● = 200 mg/L, and ▲ = 300 mg/L.

tion of a bottom-fermented lager beer, the base feed contained ethanol (4% w/w) and distilled water. The samples were filtered through the 0.45-µm membrane and through a Kieselguhr layer.

Effect of Barley β-Glucan on Filterability

To investigate the effects of β-glucan on the filtration behavior, solutions containing a middle molecular fraction of barley β-glucan in a concentration between 0 and 300 mg/L were filtered using the conditions previously described. This middle molecular barley polymer standard (M_W = 262 kDa) represents the typical molecular sizes present in beer (13,20). Compared with the base feed, the β-glucan-containing solution resulted in a decrease of filterability in membrane filtration (Fig. 1). The sample without glucan content showed a continuous filtrate flux decrease. After an increase in filtrate volume as a function of polymer concentration, the filtrate flow declined rapidly, indicating layer formation on the membrane surface and pore plugging of β-glucans in the pores. Similar effects in the context of proteins and biopolymers could be shown by several research groups (1,16,34,52). A concentration of β-glucan of 300 mg/L resulted in the lowest filterability. In this case, the flow rate decreased from 600 kg/(h × m^2) for the base feed solution to less than 400 kg/(h × m^2) for the glucan-containing solution. After 1 min of filtration time, the submitted volume was filtered through the PES membranes.

Compared with the membrane filtration, Kieselguhr filtration shows only small changes for β-glucan concentrations between 0 and 300 mg/L. The filtrate flux of these polymer concentrations was located constantly between 400 and 600 kg/(h × m^2). Furthermore, no concentration-dependent decrease of filtration speed could be observed. The filtration profile is mapped in Figure 2. Here, the cake formation seems to have a greater influence on the flow rate than the concentration of the dissolved polysaccharides. The Kieselguhr filtration represented the worst results at a concentration of 300 mg/L.

Based on these results, an effect of dissolved polysaccharides during membrane filtration could be illustrated. The filtrate volume declined with increasing β-glucan concentration. A low concentration (<200 mg/L) of polysaccharides seems to affect filterability in neither membrane nor Kieselguhr filtration. Only high concentrations of the polymer (greater than 200 mg/L) lowered the filter performance. Similar results were found by other authors (20,39). Furthermore, effects of molecular sizes of the polymers and the addition of ethanol (5 to 10% v/v) could be observed (20).

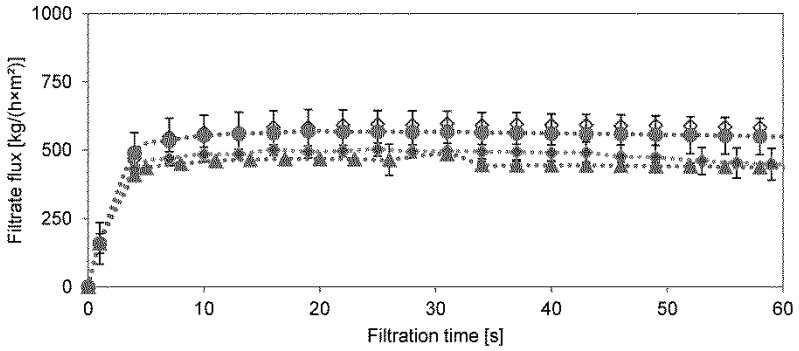

Fig. 2. Average filtrate flux (n = 3) of the Kieselguhr filtration profile of synthetic polysaccharide model solution containing barley β-glucan in an amount between 0 and 300 mg/L. β-Glucan concentration of the solution: ◇ = 0 mg/L, ◆ = 100 mg/L, ● = 200 mg/L, and ▲ = 300 mg/L.

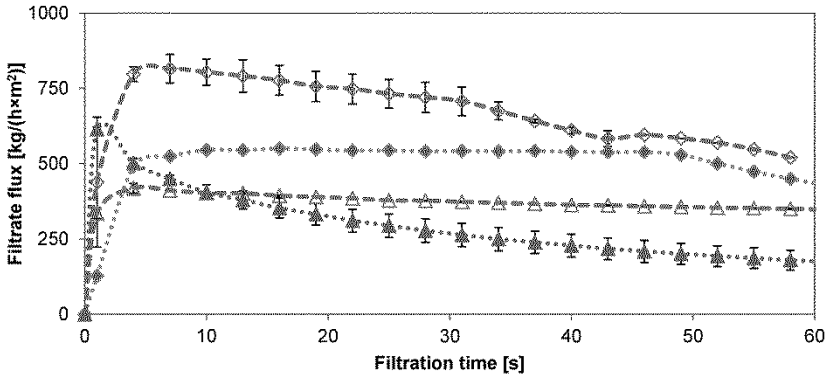

Fig. 3. Average filtrate flux (n = 3) of the microfiltration profile using polyethersulfone membranes of synthetic polysaccharide model solution containing barley β-glucan in an amount of 100 and 300 mg/L as well as medium-chain fatty acid (MCFA) ethyl esters and isoamyl acetate. β-Glucan concentration of the solution: ◇ = 100 mg/L, ◆ = 100 mg/L + esters, △ = 300 mg/L, and ▲ = 300 mg/L + esters.

Although β-glucan represents a very well-studied polymer in connection with filterability, no explanation for occasional filtration problems can be given in this context. The illustrated results and evidence from literature indicate reactions among the polymers and other beer ingredients.

TABLE I
Average Percent Changes of the Filtrate Mass for Synthetic Polysaccharide Model Solution Containing Barley β-Glucan[a]

	Change in Filtration (%)	
Solution, amount	**Membrane**	**Kieselguhr**
Barley β-glucan, 100 mg/L	+1	–23
Barley β-glucan + esters, 100 mg/L	–13	–17
Barley β-glucan, 200 mg/L	–5	–6
Barley β-glucan + esters, 200 mg/L	–30	–15
Barley β-glucan, 300 mg/L	–30	–25
Barley β-glucan + esters, 300 mg/L	–65	–42

[a] Changes of the filtrate mass after 60 s of filtration time for synthetic polysaccharide model solution containing β-glucan in an amount between 100 and 300 mg/L, as well as medium-chain fatty acid methyl esters and isoamyl acetate, calculated compared with the water-ethanol-flow.

Effects of β-Glucan and MCFA Ethyl Esters on Filterability

In addition to polymeric ingredients, different aroma compounds also can be found in beer. To investigate their effect on filtration, four different MCFA ethyl esters as well as isoamyl acetate were added to the feed base. To understand correctly the effect of each variable, filtration experiments without the addition of polymers were carried out. The aroma compounds showed no effect on filterability in either membrane or Kieselguhr filtration (data not shown). However, a decrease in the amount of esters could be observed, obviously fostered by the length of the fatty acid residues of the MCFA esters. Isoamyl acetate showed no decline.

Furthermore, filtration effects of the flavor substances and polysaccharides have been investigated. Therefore, different concentrations of barley β-glucan (M_W = 262 kDa) and constant concentration of MCFA ethyl esters were studied. For the trials, a constant concentration of ester solution at 0.5 g/L was dosed to the 2-L feed base. The filtration profiles of barley β-glucan containing polysaccharide at 100 and 300 mg/L during membrane filtration are shown in Figure 3. Compared with the pure β-glucan model solutions, the filter performance decreased by addition of MCFA ethyl esters. With increasing concentration of β-glucan, a

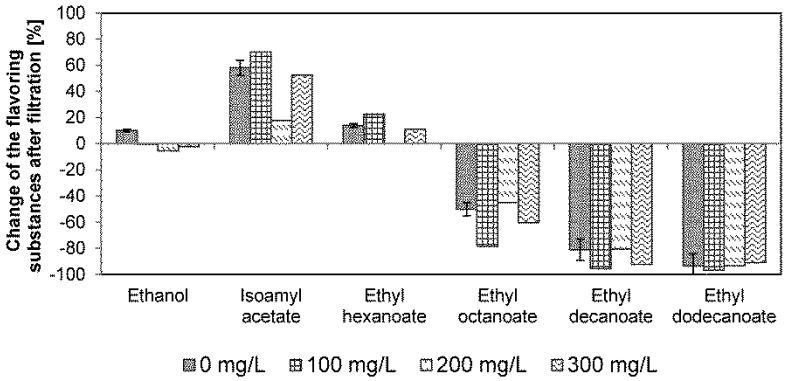

Fig. 4. Average of the percent performance of the flavoring substances after membrane filtration (n = 2) (standard error of the method 10%, error bar only shown in the first column).

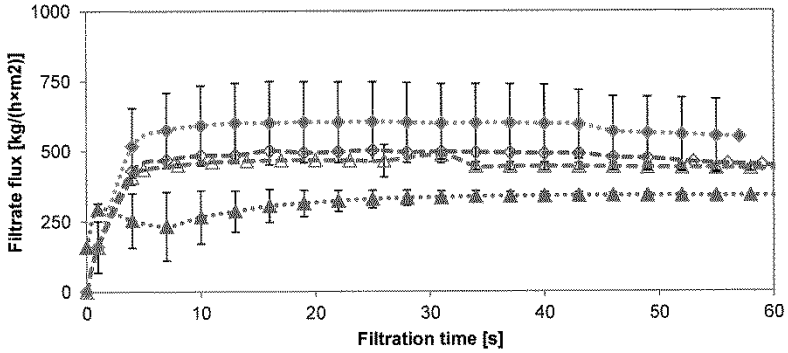

Fig. 5. Average filtrate flux (n = 3) of the Kieselguhr filtration profile of synthetic polysaccharide model solution containing barley β-glucan in an amount of 100 and 300 mg/L as well as medium-chain fatty acid (MCFA) ethyl esters and isoamyl acetate. β-Glucan concentration of the solution: ◇ = 100 mg/L, ◆ = 100 mg/L + esters, △ = 300 mg/L, and ▲ = 300 mg/L + esters.

drop in filterability could be measured. The percent reduction during all membrane filtration trials can be found in Table I. The filtrate flux changed with β-glucan at 300 mg/L from 400 kg/(h × m^2) after 60 s to less than 250 kg/(h × m^2), representing a decrease of 65% compared with the water-ethanol-flow. Using these results, a negative influence on membrane filtration with barley β-glucan concentration greater than 200 mg/L could be observed. A change of the solvent composition by the addition of flavoring substances causes a further reduction of the filtrate volume.

In contrast to the filter performance, the changes in volatiles' spectra were measured using SPME and GC analysis. This method provides a quick possibility to compare samples among themselves. According to the results shown in Figure 4, a decrease of the MCFA ethyl esters could be observed. Ethyl hexanoate showed no effect on the filtration similar to isoamyl acetate. The calculated percent increase of these two aroma compounds can be explained because of the distribution equilibrium in the headspace and the concentration-dependent accumulation on the SPME fiber (35,36). With increasing fatty acid residue chain length (C_8, C_{10}, and C_{12}), the content of MCFA ethyl esters decreased. The β-glucan concentration showed no correlation to the retention of MCFA ethyl esters.

In the second step, the model solution was filtered through a Kieselguhr layer. The filtrate flux in relation to the β-glucan concentration (100 and 300 mg/L) is shown in Figure 5. With increasing polysaccharide concentration, the filtrate flux declined (Table I). The Kieselguhr filtration resulted in a reduction of filter performance by adding MCFA ethyl esters to the polysaccharide model solution. Because of the dosage of a body feed in precoat filtration, the filtrate flux does not decrease but remains at a constant level. The β-glucan concentrations between 100 and 200 mg/L showed nearly the same effect on filterability (approximately 20% reduction). This represented a difference compared with the membrane filtration, wherein, with increasing polysaccharide content, a drop in filterability was recorded. Thus, the effect of cake formation initially has a greater influence on the filtration behavior than the ingredients of the model solution. Only a concentration of 300 mg/L affected the beer filtration.

The results of the aroma profiling yielded similar values compared with the membrane filtration (Fig. 6). However, a greater

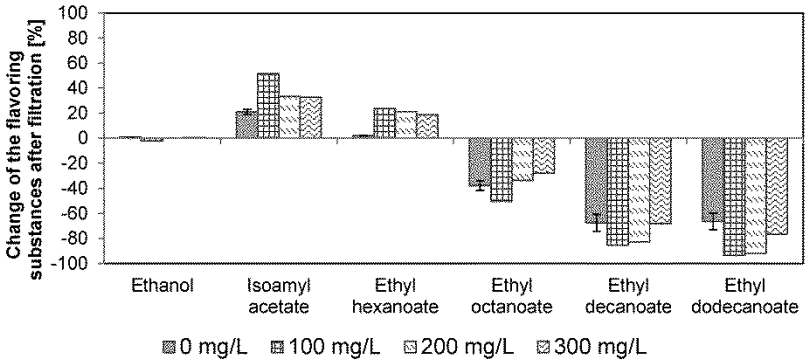

Fig. 6. Average of the percent performance (n = 2) of the flavoring substances after Kieselguhr filtration (standard error of the method 10%, error bar only shown in the first column).

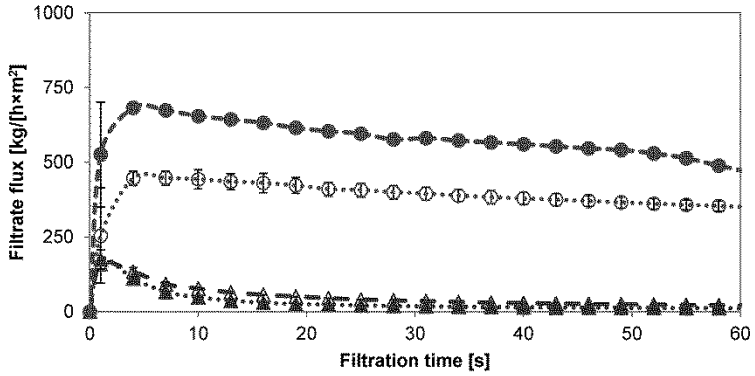

Fig. 7. Average filtrate flux (n = 3) of the microfiltration profile using polyethersulfone membranes of synthetic polysaccharide model solution containing barley and yeast β-glucan in an amount of 200 mg/L as well as medium-chain fatty acid (MCFA) ethyl esters and isoamyl acetate. Concentration and origin of the β-glucan in solution: △ = 200 mg/L (1,3;1,6)-β-glucan + MCFA esters, ▲ = 200 mg/L (1,3;1,6)-β-glucan, ● = 200 mg/L (1,3;1,4)-β-glucan + MCFA esters, and ○ = 200 mg/L (1,3;1,4)-β-glucan.

decrease of the MCFA ethyl ester could be observed in membrane filtration. In particular, the aroma-imparting ester ethyl octanoate (24%) showed a significant decline. Additionally, the difference in average decrease of ethyl decanoate (12%) and ethyl dodecanoate (6%) during membrane and Kieselguhr filtration was much lower. These esters are often absent in this type of beers (40). The low retention of acetate esters was also shown by Eagles et al. (10). Therefore, it is plausible to assume that acetate esters produce only a small effect on filtration.

In addition to cereal β-glucan, beer could also contain β-glyosidic bonded polysaccharides from yeast and bacteria. By lysis of the cells, not only flavoring substances but also other compounds such as glycogen or mannan of the yeast cell wall are released into beer and may affect the filtration (24,41). A further component of the yeast cell wall is β-glucan (21). For a comparison of the effects of the different β-glucans, filtration trials were performed. The filtration profiles for the PES membrane filtration of yeast β-glucan and barley β-glucan at 200 mg/L are shown in Figure 7. Further data for membrane and Kieselguhr filtration are mapped in Table II. The results showed a high influence of yeast β-glucan on filterability. A comparable concentration of the polymer originating from the yeast cell wall allowed almost no filtration. Also, the addition of MCFA ethyl esters and isoamyl acetate did not change the filtration.

During Kieselguhr filtration, the negative effects of yeast β-glucan also could be observed, whereas, with increasing content, a decline in filtrate flow could be shown. A concentration of yeast β-glucan of only 100 mg/L resulted in a nearly total blockage of the filter membranes. Due to the bad filtration results, no concentration dependence could be shown.

Considering these data, yeast β-glucan could have molecular sizes smaller than or comparable with the used barley β-glucan. Thus, the molecular size allows no conclusion regarding the filtration effects of yeast β-glucan. Due to the smaller particle size, it is evident that yeast β-glucan causes a blockage of membrane filter pores (7,47). Furthermore, different filtration properties can be expected due to the various molecular structures in the dissolved state, wherein yeast β-glucan has a branched helix structure and barley β-glucan has a random-coil structure (12,14,22,30). Also, in brewing industry, no limit values for the yeast β-glucan concentration in beer could be stated, because of the missing measurement methods. With the applied methods, it is only possible to characterize cereal (1,3;1,4)-β-glucan in beer (17,50). The investigated volatile compounds are formed during yeast fermentation (40). Long-chained esters are released into the beer because of lysis of yeast cells (53). A retention of aroma substances is implausible due to their low molecular weight (isoamyl acetate = 130.18 g/mol, ethyl hexanoate = 144.21 g/mol, and ethyl dodecanoate = 228.37 g/mol) (38). The results indicate interactions between the beer ingredients and the filter materials. According to Linemann (31), the size of β-glucan associates in beer is dependent on the different beer ingredients. Polar substances show an especially strong tendency to form associates. In contrast, substances which influence the polarity of the water result in a decrease of these agglomerates. During investigations of the aroma compounds and their volatility in model wine, interactions between ethyl esters and cell wall polysaccharides of yeast could be found. Ethyl octanoate, the studied component with the highest hydrophobic behavior, was found to bind in considerable quantities on yeast cell walls (32). Furthermore, they could show that the extent of binding increased with the hydrophobic nature of the esters. This statement is consistent with our data, whereupon longer-chained ethyl esters are kept back more strongly. Influences of different macromolecules and aroma compounds also have been confirmed by other authors (9,48).

TABLE II
Average Percent Changes of the Filtrate Mass for Synthetic Polysaccharide Model Solution Containing Yeast β-Glucan[a]

	Change in Filtration (%)	
Yeast β-glucan	**Membrane**	**Kieselguhr**
100 mg/L	–96	–81
200 mg/L	–98	–91
300 mg/L	–94	–94

[a] Changes of the filtrate mass after 60 s of filtration time for synthetic polysaccharide model solution containing β-glucan in an amount between 100 and 300 mg/L, calculated compared with the water-ethanol-flow.

CONCLUSION AND OUTLOOK

In this work, the behavior of β-glucan and flavoring substances during PES membrane and Kieselguhr filtration were investigated. The experiments were performed using model solutions to exclude other influencing factors occurring in beer. The dead-end filtrations were conducted at 5 °C and 1 bar pressure. The studied components included distilled water, ethanol, barley and yeast β-glucan, isoamyl acetate, ethyl hexanoate, ethyl octanoate, ethyl decanoate, and ethyl dodecanoate in various concentrations.

Membrane filtration, especially, showed a decrease in filterability, whereas higher barley β-glucan concentrations resulted in worse filtration behavior. The Kieselguhr filtration was almost not affected by barley β-glucan. Although a wide molecular weight distribution is possible in beer, the applied standard matched the filtration performance of β-glucan polymers. The addition of different esters to the barley β-glucan solution caused a stronger decrease in the filtrate flow. In particular, a concentration of barley β-glucan and esters of 300 mg/L showed a large decrease in membrane filtration. Also, during Kieselguhr filtration, a decrease in filter performance could be observed. Similar results were found using polyamide membranes. Cellulose nitrate membranes, however, showed little effect after the addition of flavoring substances.

Furthermore, a comparison of barley β-glucan and yeast β-glucan was investigated. The results show a high decrease in filterability by the addition of cell wall polysaccharides of yeast during membrane filtration. A further decrease of the filtrate flux could not be measured by the addition of aroma compounds because the membrane was blocked shortly after the beginning of the dead-end filtration.

For further investigations, it will be important to obtain information about the interactions between the aroma substances and the β-glucan occurring in beer. To observe these effects of the solvent composition on the polysaccharide structure, molecular analysis using field-flow fractionation have to be performed. Furthermore, the blocking of the membranes has to be analyzed with imaging techniques (e.g., confocal laser-scanning microscopy) for a better understanding of the fouling processes. Although a qualitative method for the differentiation of the two β-glucan sources is available (8), a method for the quantification of yeast β-glucan in beer should be created in order to obtain a more accurate statement of the unfiltered beer.

ACKNOWLEDGEMENTS

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3.4 Impact of flavouring substances on the aggregation behaviour of dissolved barley β-glucans in a model beer

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Impact of flavouring substances on the aggregation behaviour of dissolved barley β-glucans in a model beer

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ABSTRACT

Structural polymers such as cereal β-glucan may cause various processing problems in beverage industry depending on concentration, molar size distribution and agglomeration behaviour. In this context, influences of the beer volatiles dodecanoic acid, octyl butanoate, ethyl decanoate and decyl acetate on molar mass and radii of barley β-glucan were investigated in ethanolic (4% w/w) model solution. After addition of 100 mg/l ethyl decanoate and decyl acetate to the β-glucan solution, a wider-ranging molar mass distribution could be observed by means of asymmetric field-flow-fractionation. Due to agglomeration, average molar mass of β-glucan standard ($M_W = 6.8 \times 10^6$ g/mol) increased by 2×10^6 g/mol ($P < 0.05$) in solution containing decyl acetate. Furthermore, a significant growth ($P < 0.05$) from 86 to 102 nm in gyration radius was measured. The obtained results elucidate the importance of fatty acid derived flavouring substance composition in beer regarding the aggregation behaviour of β-glucan.

1. Introduction

Polysaccharides are a key component of virtually all foods and beverages. Depending on the structure and composition, these polymers are important for the nutritional, health and taste value of foods such as pasta or bakery products as well as beverages like beer (Anttila, Sontag-Strohm, & Salovaara, 2004; Sahan, Yasar, & Hayaloglu, 2008). Besides texture and appearance polysaccharides could modify the rate and intensity of flavour molecule release due to specific and non-specific interactions (Boland, Buhr, Giannouli, & van Ruth, 2004; Shin, Lee, Chang, Lee, & Kim, 2014). The nutritional and health value is influenced by the amount of soluble fibres in the foods (Collins et al., 2010). Such fibres exist in beer in form of β-glucans, arabinoxylans and other polysaccharides resulting from barley malt as main source (Sadosky & Schwarz, 2002; Tiwari & Cummins, 2011). The β-glucans are (1→3)(1→4)-β-D-linked linear chains and occur in barley in a concentration between 3% and 10% (Nielsen, Karlsson, & Engelsen, 2008). Because this β-bond is not digestible by enzymes in human gastrointestinal tract, these polysaccharides have a moderating effect on postprandial blood glucose, insulin response and reduce elevated blood cholesterol levels (Burkus & Temelli, 2005; Sahan et al., 2008). Although, β-glucans have a positive effect on human metabolism, the processing of glucan-containing raw materials often results in various problems because of its viscoelastic properties. Especially in terms of beer filtration β-glucan is a well-known substance, whereas the presence of higher concentrations yields in a decline of filterability (Bamforth, 1982; Kreisz, Spieleder, & Back, 2003). Particularly high molar fractions (>10^5 g/mol) are known to have a negative impact on beer filtration (Jin, Speers, Paulson, & Stewart, 2004b; Stewart, Hawthorne, & Evans, 1998). The molar mass distribution of β-glucans in beer depends, e.g., on differences in raw materials, malt modification, action of native enzymes, milling process, mashing intensity as well as succeeding manufacturing processes (Grimm & Krüger, 1995; Manzanares, Navarro, Sendra, & Carbonell, 1991; Marconi, Tomasi, Dionisio, Perretti, & Fantozzi, 2014). Marconi et al. (2014) showed a decreasing molar mass during malting depending upon germination time of barley. During mashing, these malt β-glucans are released by enzymatic and thermal hydrolysis in a molar mass range between 10^3 and 10^7 g/mol (Anderson, 1990; Bamforth & Martin, 1983; Foldager & Jørgonson, 1984). Comparable molar size area distributions of β-glucans were also detected in beer (Grimm & Krüger, 1995; Manzanares et al., 1991). Cereal β-glucans exhibit the ability to form gels, an association or cross-linking of polysaccharide chains via hydrogen bonds to form a 3-dimensional network (Burkus & Temelli, 1999; Clasen & Kulicke,

Abbreviations: AsFiFFF, Asymmetric flow-field-flow-fractionation; HMM, High molar mass; log *P*, partition-coefficient; LS, Light scattering; MALLS, multiangle laser light scattering; MCFA, Medium chain fatty acid; RI, refractive index; x_M, degree of association; x_r, aggregation number; ρ, ρ-parameter.

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2003; Tosh, Wood, & Wang, 2003; Vaikousi, Biliaderis, & Izydorczyk, 2004). These agglomerates have a negative impact on filtration due to their augmented particle sizes (Fischer, 2005). Besides enhancing effects of shear forces and reaction time, an influence of low sugar and high ethanol concentrations as well as low temperatures could be described on gel formation (Grimm, Krüger, & Burchard, 1995; Tosh et al., 2003; Ulmius, Önning, & Nilsson, 2012; Vaikousi et al., 2004). Other effects regarding agglomeration could be observed in terms of volatility of several flavour substances (Voilley, Lamer, Dubois, & Feuillat, 1990). The addition of β-glucans resulted in a lower volatility of aroma compounds which could be shown to be declining with increasing lipophilicity (log*P* value) of the aroma compound in question (Christensen, Trindade Leitão, Petersen, Jesperson, & Engelsen, 2009; Shin et al., 2014). Similar results were observed during filtration of beer model solutions, where a decrease of flavouring substances with rising log*P* value could be measured (Kupetz, Zarnkow, et al., 2015). In addition to the loss of aroma substances filterability was impaired. These flavour compounds are in addition to acetate esters (e.g., ethyl acetate and isoamyl acetate) also medium-chain fatty acid (MCFA) ethyl esters, formed during alcoholic fermentation (Procopio, Qian, & Becker, 2011). In alcoholic beverages, these esters carry fatty acid residues between C_6 and C_{12}, whereas their concentrations differ with respect to yeast fermentation performance (Jiang & Zhang, 2010; Saerens, Verstrepen, Thevelein, & Delvaux, 2008; Suomalainen, 1981).

Although, structure and agglomeration behaviour of β-glucans are abundantly studied to date, none of the authors referred to influences of hydrophobic substances in beer in this context. However, the described influences of β-glucan on volatile release and negative effects on filterability suggest on structural impact on the polysaccharide. The aim of this research is the investigation of changes in molecular shape, agglomeration behaviour and viscosity of dissolved barley β-glucan affected by hydrophobic volatiles. To exclude further beer ingredients that may affect the examination, model beer containing barley β-glucan and different volatiles (free fatty acid and fatty acid derived flavouring substances) were chosen. Furthermore, the log*P* value should be validated as criterion for interactions with β-glucans, which would provide important information for several processing steps, particularly regarding the performance of beer filtration.

2. Material and methods

2.1. Materials

(1,3;1,4)-β-D-Glucan from barley (high viscosity barley β-glucan, Megazyme International Ireland, Dublin, M_W: 4.95×10^6 g/mol, purity: >94% (dry weight basis)) from one batch was used to investigate the influence of polysaccharides in beer because of its broad molar size distribution. A molar mass of 4.95×10^6 g/mol was reported by manufacturer, measured using size-exclusion chromatography combined with multiangle laser light scattering (MALLS) in a solution containing 1 mg/ml polysaccharide, 0.1 M sodium nitrate and 5 mM sodium azide (Megazyme, 2013). To study the effects of hydrophobic substances on the structure of β-glucans, dodecanoic acid (Fluka GC reference 61609, Fluka Analytical, Switzerland), octyl butanoate (Sigma, MKBG 5186V, Sigma-Aldrich Co. LLC., Germany), ethyl decanoate (Fluka 13404930K, GC reference, Fluka Analytical, Switzerland) and decyl acetate (Schuchardt, Merck Schuchardt OHG, Germany) were tested. The physicochemical data of the aroma compounds are shown in Table 1. These substances were chosen because of a similar hydrophobic constant (log*P* value) between 4.96 and 5.03 and a flash point above 100 °C. In addition, absolute ethanol from Merck (Germany) was used to simulate the beer composition.

For preparation of the beer model solutions, 1 g/l of the powdered β-glucan was weighted and diluted in 0.5 kg double distilled water. The suspension was heated on a hot plate until boiling. The temperature was maintained for 30 min. The complete solubility of β-glucan was checked visually. After cooling to 20 °C, the polysaccharide stock solution was weighed to 1 kg with double distilled water. All analysis solutions contained 4% (w/w) ethanol. Ethanol concentration was chosen due to usual occurring contents in bottom-fermented beer types (Krüger & Anger, 1990). Polysaccharide content was deliberately chosen in upper region of occurring concentrations in beer to allow an analysis with field-flow-fractionation (Jin, Speers, Paulson, & Stewart, 2004a). The flavouring substances were added in a concentration of 50 and 100 mg/l to β-glucan stock solution. These concentrations were chosen because of the total lipid amount in unfiltered and filtered beer (Bravi, Perretti, Buzzini, Della Sera, & Fantozzi, 2009). The final samples had a slight opalescence. The pH values of the samples varied between 3.6 and 4.2 and thus were close to the beer-pH.

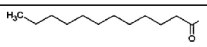
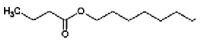
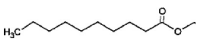
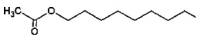
2.2. Viscosity measurement

Since β-glucan is known to influence beer viscosity due to concentration, molar mass and gel content, viscosity measurement was performed using a rotational viscometer (Stabinger viscometer SVM 3000, Anton Paar, Graz) at 20 °C according to Analytica-EBC analysing methods (Jin, Speers, Paulson, & Stewart, 2004c; Welten, 2013). 12 ml of beer model solutions were filled in a test tube, measurement cell got pre-wetted with sample and dynamic viscosity (η) as well as density was determined in triplicate. Method is based on torque and speed measurement of a rotating magnet in SVM 3000. Three cycles air injection for 200 s were used for measurement cell cleaning after each sample. Density was determined by means of an integrated density measurement cell based on an oscillating U-tube system. Sample was automatically drawn into the U-shaped test tube and caused to oscillate. Measured oscillating period corresponded to sample density. Influence of temperature was compensated by precise temperature measurement.

2.3. Determination of β-glucan and β-glucan-gel content

Among detection of fluorescence intensity due to interactions between β-glucans and Calcofluor, influences of hydrophobic substances on β-glucan concentration measurement should be investigated applying Analytica EBC method 9.31.2 (Welten, 2013). This method is based on interactions between the dye Calcofluor and β-glucan due to hydrogen bonds, ionic interactions and vander-Waals forces (Wu, Deng, Tian, & Xie, 2008). The microtitre assay was accomplished using calibration standard of SBL (Scandinavian Brewery Laboratory Ltd., Copenhagen) with a concentration of 500 mg/l β-glucan. Initially, 15 µl of the standard was transferred into a 96-well plate by means of pipetting robot BioTek Precision XS (BioTek Instruments, Inc., Winooski United States) to create a 7-point calibration. 300 µl dye solution containing 5 ml Calcofluor (Sigma) and 495 ml degassed Tris-HCl buffer (0.1 mol/l pH 8.0) were pipetted into each cavity of the 96-well plate. The 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 calculation of β-glucan content of the model beer 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. Because of the initial weight, all samples were diluted 1:3 before measurement with double distilled water. Samples were

Table 1
Physiochemical data of the aroma compounds.

Aroma compounds	Structural formula	Molar mass [g/mol][1]	Flash point [°C][1]	log P[1]	Water solubility [mg/l][1] at 25 °C
Dodecanoic acid		200.32	134.1 ± 11.9	5.03	12.76
Octyl butanoate		200.32	103.3 ± 0.0	4.96	3.52
Ethyl decanoate		200.32	102.2 ± 0.0	4.96	3.52
Decyl acetate		200.32	101.3 ± 6.9	4.96	3.52

[1] Advanced Chemistry Development, Inc., ACD/labs (www.chemspider.com).

prepared and measured in same way as calibration standards were done.

β-Glucan gel content was determined according to Krüger, Wagner, and Esser (1989). The method is based on the break-down of hydrogen bonds between agglomerated β-glucans via heat exposure, which allows a dye reaction of separated β-glucan chains with Calcofluor (Kupetz, Procopio, et al., 2015). One part of each beer model sample was heated to 80 °C for 20 min. The concentration differences between heated and non-heated samples are corresponding to gel-content. All measurements were performed in quadruplicate.

2.4. Instrumental set-up and procedure of the AsFlFFF

The structural properties of different β-glucan samples, prepared according to Section 2.1, were determined by asymmetrical flow field-flow-fractionation (AsFiFFF; Wyatt Technology, Germany) coupled with MALLS (Dawn, Heleos II, Wyatt Technology, Germany) and refractive index (RI) as quantitative detector (Agilent Series 1200 G1362A, Agilent Technologies, Germany) (Rübsam, Gastl, & Becker, 2013). The described system was used to determine weight-average molar mass (M_W), dispersity, root mean square radius (r_{rms}), the exponent of the confirmation plot υ as well as hydrodynamic radii (r_h).

In order to detect impact of solvents (water/ethanol or water/ethanol/volatile) on agglomeration behaviour and solubility of β-glucans, a carrier solvent consisting of sodium nitrate (50 mM, Merck) and sodium azide (250 ppm, Sigma) in double distilled water (Li, Wang, Cui, Huang, & Kakuda, 2006; Ulmius et al., 2012) was chosen. This is a common method to prevent polysaccharide aggregation during field-flow-fractionation measurement targeting M_W detection. Furthermore, direct use as solvent prevents polysaccharide aggregation (Li et al., 2006; Li, Cui, Wang, & Yada, 2011). Measurement was performed at 25 °C. An auto sampler (1200 Series, Agilent Technologies) handled sample injection. As accumulation wall a membrane from regenerated cellulose with a cut-off of 5×10^3 g/mol (Microdyn Nadir GmbH, Wiesbaden, Germany) was used. The inserted spacer in the flow-channel had a height of 350 μm, a width of 21.5 mm at the widest position and a channel length of 240 mm. Aliquots of 100 μl beer model sample prepared according to Section 2.1 were injected at a rate of 0.2 ml/min. Before elution, sample was focused on membrane, in order to achieve a size-dependent accumulation on membrane surface (Ulmius et al., 2012). Various focus times were tested using aqueous β-glucan standard solutions. Focusing time of 8 min was chosen for all other experiments. The elution flow was 1.0 ml/min and crossflow decreased from 4 to 0.2 ml/min within 25 min. At the end, the crossflow was finally reduced to zero within 10 min. The detector flow was constant at 1.0 ml/min throughout separation.

2.5. Calculations

The analysis of the light scattering data was performed using Astra software (version 6.1.2.84, Wyatt Technology). Baseline corrections for RI signal were subtracted by blank baseline subtraction from a blank run containing only carrier liquid (Ulmius et al., 2012). The results described in section before were calculated using Zimm-plot for 34.7–142.5° scattering angles. Higher and lower scattering angles were not included. According to Li et al. (2011), the RI increment, dn/dc was set to 0.1460 ml/g for β-glucan in aqueous solution. Band broadening was reduced by detector calibration with BSA standard (Sigma-Aldrich Co. LLC., Germany) (Podzimek, 2011).

Validity of field-flow-fractionation system was tested analysing medium molar mass (M_W) of β-glucan standards (low/medium/high viscosity β-glucan standards, Megazyme International Ireland, Dublin) in comparison to manufacturer data. Here, a maximum deviation of less than 1% was determined.

2.6. Statistics

Statistical analysis was carried out using OriginPro 2015G (OriginLab Cooperation, Northampton, USA). To compare differences between the values a one-way ANOVA with fisher-test LSD using significance level (α) 0.05 was calculated.

3. Results and discussion

For determination of the impact of flavouring substances on β-glucans agglomeration behaviour initially viscosity of the samples was determined. The dynamic viscosity related to β-glucan concentration of control sample could be investigated as 25.82 ± 0.19 (mPa × s)/mg ($n = 3$). The addition of flavouring substances resulted in a change in viscosity related to β-glucan concentration in sample, shown in Table 2. In dependence to the added flavour compound and concentration the samples showed significant differences ($P < 0.05$) to the β-glucan control solution without flavouring substances. In particular, the decyl acetate samples had a significant lower dynamic viscosity (η/c_{50} mg/l = 21.68 ± 0.71 (mPa × s)/mg, $n = 3$, η/c_{100} mg/l = 23.45 ± 0.32 (mPa × s)/mg, $n = 3$). A viscosity increase compared to control sample could be observed in the samples containing dodecanoic acid (η/c_{50} mg/l = 27.27 ± 1.62 (mPa × s)/mg, $n = 3$). The results of viscosity measurement show clear influences of amount and type of volatiles on β-glucans in solution.

Table 2
Averages of dynamic viscosity $\eta_{solution}$ in relation to β-glucan content ($n = 3$) as well as the β-glucan-gel content c_{gel} ($n = 4$).

	Concentration volatiles [mg/l]	$\eta_{solution}/c_{\beta\text{-glucan}}$ [(mPa × s)/mg]	c_{gel} [mg/l]
Control	0	$25.82^{a,f}$	4 ± 4
Dodecanoic acid	50	$27.27^{d,e}$	0 ± 6
Octyl butanoate	50	$24.66^{c,e}$	7 ± 7
Ethyl decanoate	50	25.94^{b}	4 ± 1
Decyl acetate	50	$21.68^{a,b,c,e}$	8 ± 2
Dodecanoic acid	100	25.52^{i}	0 ± 1
Octyl butanoate	100	25.73^{h}	0 ± 0
Ethyl decanoate	100	25.31^{g}	9 ± 4
Decyl acetate	100	$23.45^{f,g,h,i}$	12 ± 2

Mean values in the same column followed by the same letter are significantly different ($P < 0.05$).

Effects on viscosity could be explained in dependence to molar mass of polymers, their concentration as well as their aggregation behaviour (Gómez, Horta, & Carbonell, 1997; Ulmius et al., 2012). In particular, the significantly lower viscosity related to β-glucan content after adding decyl acetate was conspicuous. Furthermore, β-glucan content as well as the β-glucan-gel concentration were determined using a fluorometric assay. Noticeable was a lower measurable β-glucan concentration by means of fluorometric assay compared to weighted concentration (data not shown). No significant differences ($P > 0.05$) could be observed, neither in β-glucan concentration nor in gel content of the different beer model solutions, although an increased gel content in the samples with ethyl decanoate ($c_{gel,\ 100}$ mg/l = 9 ± 4, $n = 4$) and decyl acetate ($c_{gel,\ 100}$ mg/l = 12 ± 2, $n = 4$) was measured. This detected gel concentrations could not arise because of shear forces or cold storage of the samples. Influences of solvent composition for instance of ethanol, pH value and temperature are well described in literature (Jin et al., 2004c). Impact of flavouring substances on β-glucan solubility that resulted in changes in molar mass, gel content or viscosity could not be found in literature. In order to identify the impact of the investigated volatiles on β-glucan molar size distribution and further particle properties, analysis using field-flow-fractionation combined with MALLS detector were performed.

Typical fractograms, showing RI signal (directly proportional to the concentration) and LS signal in the same plot, demonstrated no differences in concentration within all samples (see Fig. 1). LS signal had slight changes in samples with addition of 100 mg/l dodecanoic acid and octyl butyrate in comparison to control sample (see Fig. 1A). Higher influences of LS signals could be found in samples containing 100 mg/l ethyl decanoate or decyl acetate (see Fig. 1B).

Average molar mass M_W of the pure β-glucan ethanolic solution could be determined as 6.8×10^6 g/mol (range of M_W $6.6–7.2 \times 10^6$ g/mol, $n = 3$). The addition of aroma compounds resulted only in slight changes in molar mass distribution (see Fig. 2A). In particular, the sample with decyl acetate had an altered distribution portion between 1×10^5 and 1×10^6 g/mol. Above 1×10^6 g/mol addition of ethyl decanoate and octyl butyrate resulted in a change of molar mass distribution in comparison to control sample. Dosage of the volatile dodecanoic acid had lowest impact on cumulative weight fraction distribution of molar mass of β-glucan.

The investigation of 100 mg/l volatiles combined with β-glucan resulted in a wider-ranging molar mass distribution above 1×10^6 g/mol, primarily for ethyl decanoate and decyl acetate, shown in Fig. 2B. The other flavour substances resulted in a similar width in comparison to control sample. In particular, addition of the free fatty acid had nearly non-effect on molar mass distribution. Especially molar mass distribution below 5×10^5 g/mol had only slight deviations due to the addition of 100 mg/l volatiles to control

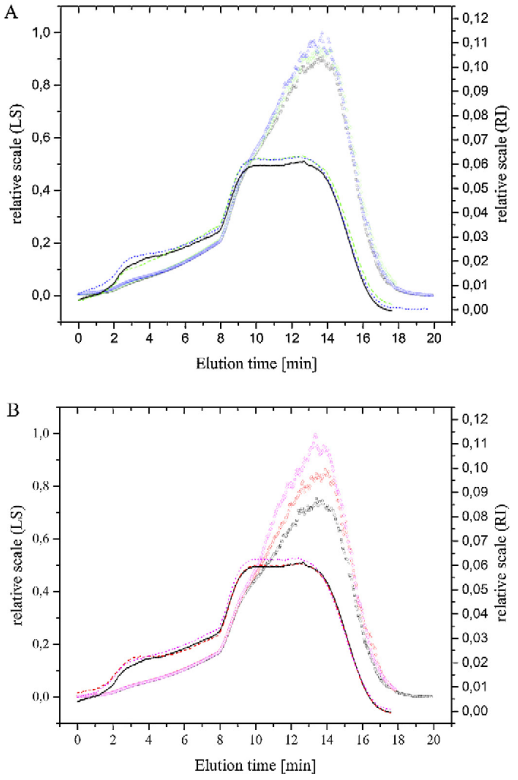


Fig. 1. Fractograms from AsFiFFF analysis of pure barley β-glucan (control (□/—)) in comparison to samples containing also 100 mg/l dodecanoic acid (◇/– –), octyl butanoate (△/.....), ethyl decanoate (○/– –) or decyl acetate (▽/.....). All samples are dissolved in distilled water containing 4% (w/w) ethanol. The left *y* axis shows MALLS (dots) and right axis the normalized RI signals (lines).

Table 3
Molecular characteristics ($n = 3$) of model beer samples with addition of 50 mg/l volatiles, quoting significant differences from β-glucan solution without the addition of flavouring substances.

	M^1 [$\times 10^6$ g/mol]	Dispersity (M_w/M_n)	$r_{rms}{}^2$ [nm]	υ^3
Control	$6.84^{a,b}$	1.8	86^{a}	0.55^{a}
Dodecanoic acid	$7.09^{e,f}$	1.8	$88^{d,e}$	0.55^{d}
Octyl butanoate	$7.93^{b,d,f}$	2.0	$98^{a,c,e}$	0.55^{c}
Ethyl decanoate	$7.81^{a,c,e}$	1.9	$96^{b,d}$	0.55^{b}
Decyl acetate	$6.76^{c,d}$	1.9	$86^{b,c}$	$0.48^{a,b,c,d}$

Mean values in the same column followed by the same letter are significantly different ($P < 0.05$).
[1] w-average.
[2] z-average.
[3] r_{rms} confirmation plot (exponent from the plot r_{rms} versus M_w).

β-glucan sample. Thus, the addition of flavouring substances to β-glucan solution yielded in major changes in molar mass distribution, whereas the impact on molar mass strongly depends on concentrations and type of hydrophobic substances. The field-flow-fractionation coupled with light scattering and refractive index detector provides the ability for determination of gyration radius and other particle properties. Table 3 gives an overview of the

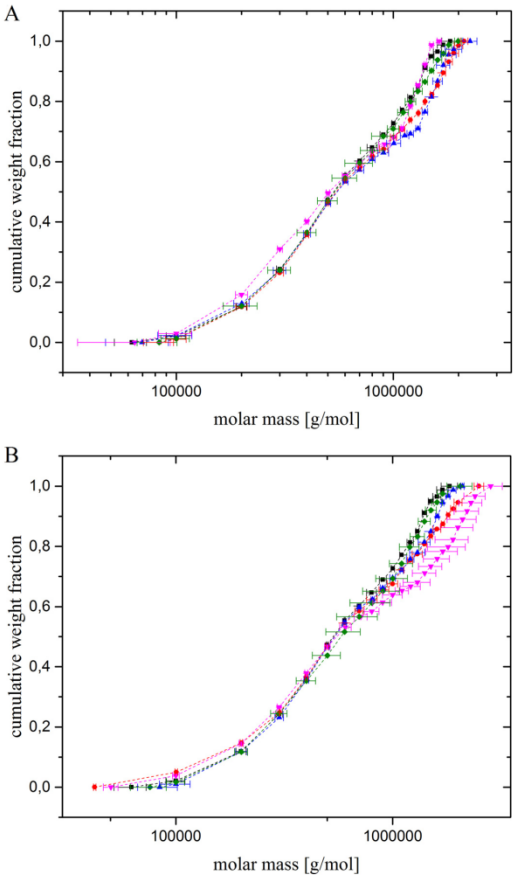


Fig. 2. Cumulative weight fraction of the molar mass [g/mol] of β-glucan solution in comparison to the addition of different hydrophobic agents in a concentration of 50 mg/l (A) and 100 mg/l (B), legend: ■-control, ● -ethyl decanoate, ▲ -octyl butanoate, ▼ -decyl acetate, ◆ -dodecanoic acid.

averages of molar mass, dispersity and radii of β-glucan solutions containing 50 mg/l volatiles in comparison to control sample.

Average molar mass (M_W) increased significantly ($P < 0.05$) from 6.8 (control) to 7.8 (ethyl decanoate) and 7.9×10^6 g/mol (octyl butanoate). The significantly higher molar mass of control sample compared to manufacturer's data has been achieved due to the use of different solvents. Furthermore, dispersity (M_n/M_W) had slight differences depending on the dosed volatile. A significant ($P < 0.05$) average rise of 10 (ethyl decanoate) and 13 nm (octyl butanoate) could be measured in gyration radius (z-average). The samples with dodecanoic acid and decyl acetate had no significant changes in detected radii. The exponent of the confirmation plot as function of molar mass (M_W) and gyration radius (r_{rms}) showed only slight variety. Majority of the samples had an exponent of 0.55, which indicates a random coil structure (Li et al., 2011). Only β-glucan with decyl acetate had a significant ($P < 0.05$) lower exponent of 0.48.

The mean values for the addition of 100 mg/l volatiles are shown in Table 4. Depending on the investigated flavouring substances

Table 4
Molecular characteristics ($n = 3$) of model beer samples with addition of 100 mg/l volatiles, significant differences from the β-glucan solution without the addition of flavouring substances.

	M^1 [$\times 10^6$ g/mol]	Dispersity (M_n/M_w)	r_{rms}^2 [nm]	υ^3
Control	6.84^a	$1.8^{a,b}$	86^a	0.55
Dodecanoic acid	7.24^c	1.8^d	89^c	0.56
Octyl butanoate	7.49^b	1.9^c	92^b	0.56
Ethyl decanoate	7.81	$2.3^{a,c,d}$	96	0.54
Decyl acetate	$9.18^{a,b,c}$	$2.5^{b,c,d}$	$102^{a,b,c}$	0.50

Mean values in the same column followed by the same letter are significantly different ($P < 0.05$).
[1] w-average.
[2] z-average.
[3] r_{rms} confirmation plot (exponent from the plot r_{rms} versus M_w).

Table 5
Aggregation numbers (x_r) (according to Li et al. (2011)), degree of association (x_M) (according to Grimm et al. (1995)) and ρ-parameter (according to Grimm et al. (1995)) in dependence to the manufacturing data of the β-glucan sample and an addition of 100 mg/l volatiles.

	x_M^1 (M/M_0)	x_r^2 (r/r_0)	ρ (r_{rms}/r_h)
Control	1.4^a	1.9^a	1.0
Dodecanoic acid	1.5^c	1.9^c	0.9
Octyl butanoate	1.5^b	2.0^b	1.0
Ethyl decanoate	1.6	2.1	1.2^a
Decyl acetate	$1.9^{a,b,c}$	$2.2^{a,b,c}$	0.7^a

Mean values in the same column followed by the same letter are significantly different ($P < 0.05$).
[1] w-average.
[2] Gyration radii.

major changes in average molar mass were measured. Addition of 100 mg/l free fatty acid had the lowest degree of influence on β-glucan structure, followed by octyl butanoate. M_W of β-glucan increased from 6.8 (control) to 7.8×10^6 g/mol ($n = 3$) by adding ethyl decanoate. The dispersity widened from 1.8 to 2.3, accompanied with the rise of the radii. The biggest impact on the width of distribution affected decyl acetate. Besides an increase in molar mass to 9.2×10^6 g/mol (range of M_W 8.3–10.7×10^6 g/mol, $n = 3$), also a larger root mean square radius (102 nm) could be observed. The exponent υ did not change significantly ($P > 0.05$), indicating a random coiled structure. Only decyl acetate had a lower value, which is consistent with the data of the viscosity measurement where also smaller values could be observed.

Furthermore, significant differences in dispersity can be determined depending on dosage amounts of volatiles. Especially ethyl decanoate and decyl acetate showed significant differences ($P < 0.05$) after the addition of 50 or 100 mg/l volatiles. Summarizing these results, it can be noted that significant variations in molar mass and particle size depending on the used volatiles can be observed.

The analysis of the used β-glucan standard of the manufacturer resulted in a molar mass $M_W = 4.95 \times 10^6$ g/mol and an average gyration radius of 45.8 nm dissolved in buffered solution containing sodium nitrate (0.1 M) and sodium azid (5 mM). Compared to the measurement results of different authors (Grimm et al., 1995; Li et al., 2006; Li et al., 2011), polysaccharide dispersions in saline solutions were used to determine the aggregation potential of particles as a function of solvent composition. Using the buffer solution consisting of sodium nitrate (0.1 M) and sodium azid (5 mM) the manufacturing data could be confirmed.

Grimm et al. (1995) as well as Li et al. (2006) presented indicators describing agglomeration potential of polysaccharides. The calculated data are shown in Table 5. In comparison to results of Grimm et al. (1995), the degree of association (x_M) is smaller, but a tendency paralleled by the addition of volatiles could be observed.

The influence of ethanol on the structure of β-glucan is considerable. Comparable results for barley β-glucans by Li et al. (2011) could be achieved by calculating the aggregation number (x_f). The author could obtain higher aggregation numbers with decreasing molar mass. In particular, due to the addition of ethyl decanoate and decyl acetate differences could be performed. Not only the aggregation potential but also structural properties of polysaccharides could be determined using measurement results of β-glucan gyration and hydrodynamic radii. This ρ-parameter is sensitive for structure of particles in solution (Grimm et al., 1995). According to Burchard (2005) and Grimm et al. (1995), a value of $\rho \cong 1.0$ corresponds to star-branched structures. Furthermore, it can be stated that investigated β-glucan solutions had a $\rho > 1$ resulting in a star-branched structure (number of arms (f) > 1), whereas the β-glucans with decyl acetate had a more spherical architecture ($\rho = 0.778$) (Burchard, 2005). Even in this case, the addition of two esters decyl acetate and ethyl decanoate resulted in a significant difference ($P < 0.05$) to control sample. This is consistent with the data of our viscosity measurements, wherein addition of decyl acetate resulted in significantly lower values. A significant correlation ($r = 0.96$, $P < 0.05$) between molecular structure υ and viscosity related to β-glucan content of the sample could be determined.

The detected variations in molar mass, particles size and structure can be related to the ability of β-glucan to form gels. This could be illustrated by an increase of molar mass as well as radii and a wider dispersity of the investigated polysaccharide solutions. The gel formation is described to depend on solvent composition and temperature (Burkus & Temelli, 1999; Grimm et al., 1995). The presence of LMW hydrophilic molecules such as sucrose or maltose lowers the level of water activity in solvent resulting in a lower ability of aggregation, whereas a maltose content of 5% resulted in best solubility of β-glucan molecules and thus lowest aggregation (Grimm et al., 1995). This influencing factor could be excluded, since no sugar was present in the chosen model solution. Yalpani (1988) proposed that a lowered water activity level provokes β-glucan polymer interchain binding. In this context the presence of ethanol promotes association of β-glucan because of a lowered dielectric constant enhancing tendency to form bonds (Jin et al., 2004a). This could be efficiently demonstrated with our results due to an impaired solubility of β-glucans in model solution. For this reason, higher average molar masses could be measured in control sample in comparison to manufacturer's data. The addition of volatiles to the solvent reinforced this effect.

Particle interactions between polysaccharides and volatiles assumed by different research groups (Dufour & Bayonove, 1999; Shin et al., 2014; Voilley et al., 1990) could be confirmed with the shown results. According to literature, especially high molar β-glucans had higher retention of flavouring substances (Christensen et al., 2009). This could be validated with the used high molar mass barley β-glucan standards on the basis of large molar growth. However, different effects of solvent composition on structure and agglomeration behaviour of the β-glucans could be measured despite of identical log P values and water solubility (see Table 1) of the investigated volatiles. No correlation between β-glucan agglomeration potential in connection to lipophilicity or water solubility could be observed. Highest water solubility of dodecanoic acid corresponded to lowest β-glucan aggregation. In contrast, differing impact on molar mass could be measured regardless identical water solubility of the other investigated volatiles. The resulted drop in water availability due to solvent modification caused an increase in agglomeration (Parker, Elmore, & Methven, 2014). The slightly occurring differences in pH value of investigated model beer solution may be influenced aggregation behaviour of β-glucans. In order to detect these effects a large pH range needs to be investigated. Initial results of influence of pH value can be found by Shin et al. (2014).

However, this contradicts literature describing clear linkages between log P value of volatiles and aroma release in polysaccharide solutions (Christensen et al., 2009; Shin et al., 2014). Though numerically almost identical in present case, it appears that log P value provides no meaningful prediction criterion. This is consistent with findings of Philippe et al. (2003) who showed that log P value and solubility in water provided no explanation for different vapour/water partition coefficients and thus the volatility of flavouring substances in beverages. The results indicate that interactions of volatiles and polysaccharides are not only depending on solubility of aroma compounds. Rather geometry and type of functional groups of flavouring substances as well as pH of the solvent ought to be considered, which is accompanied by evidence of the literature (Juteau, Tournier, & Guichard, 2004; Shin et al., 2014).

Regarding beer, this knowledge is very important because of interactions occurring among different beer ingredients. For β-glucan gels often appear spontaneously in beer, process problems cannot be detected and solved in time. These agglomerates are known to cause problems in beer filtration due to higher molar masses. Several authors demonstrated high β-glucan concentrations in beer cold break after fermentation (Annemüller, Nagel, & Bauch, 1998; Senge & Annemüller, 1995). These results demonstrated a low solubility of β-glucans in beer matrix. However, no conclusion regarding gel content, molar mass distribution or impact of further beer ingredients were not shown. Since decreases of MCFA-ethyl esters could be detected in beer filtration processes (Kupetz, Weber, et al., 2015; Kupetz, Zarnkow, et al., 2015; Walla, 1991), synergistic effects of β-glucans and beer volatiles are imaginable. Ulmius et al. (2012) as well as Gómez et al. (1997) could show a decrease in molar mass (M_W) by filtration using organic polymer membrane filter. Besides this drop in molar mass, only small changes in β-glucan concentration of the stock solutions could be observed. These results suggest a break-down of most molecular aggregates because of shear forces produced through the membrane (Gómez et al., 1997; Vårum, Smidsrød, & Brant, 1992). Furthermore, hydrophilic-hydrophobic interactions of the agglomerates consisting of β-glucan and beer flavouring substances depending on the used membrane materials are possible (Maximous, Nakhla, & Wan, 2009; van der Sman, Vollebregt, Mepschen, & Noordman, 2012). Thus, changes in microstructures in beer matrix due to agglomeration may have a major effect on the process management especially during filtration.

4. Conclusions

Based on the measured data it can be concluded that hydrophobic substances increase the agglomeration potential of β-glucan. In this case, not only influences of volatile concentration, but also on the type of flavouring reagent could be demonstrated. Since the reagents have different effects on the β-glucans, though log P value is nearly the same, this coefficient cannot be used as a characterizing value for agglomeration potential of polysaccharides. The release of aroma compounds in glucan containing foods was described by different authors, but accurate type behind the retention could not be shown. Since aroma mixtures in a wide log P range were used of the research groups and the aroma retention certainly also depends on the additional food matrix, the influence of lipophilicity could only be inadequately characterized. Influences on the molecular structure (υ, ρ) of β-glucans depending on the used flavouring substances were shown. In this context, incorporation of the volatiles into the β-glucans coils, accompanied with retention of volatility, is conceivable. However, the precise mechanisms of interaction cannot be clarified.

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3.5 Impact of fatty acids and medium chain fatty acid ethyl esters on the beer crossflow membrane filtration

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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].

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

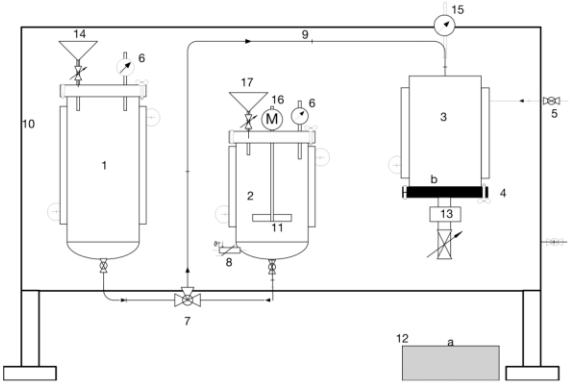

Fig. 1 **Automatic laboratory filter system consisting of a precoat vessel (1), a sample vessel (2) and a filter unit (3). A sieve with 15 µm pore size (Raible-test) or a membrane (Esser-test) could be placed (b) in this filter unit. The filter is equipped with a cooling unit (5), a CO_2-connections (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.

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

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

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.

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

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

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

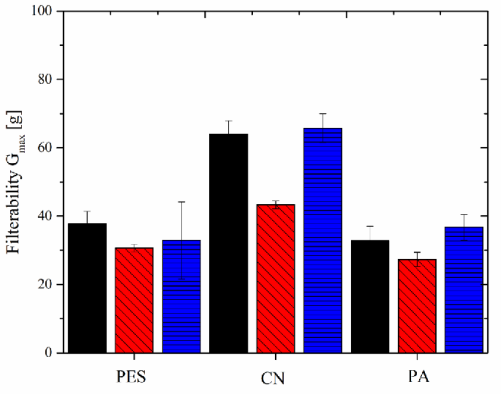

Fig. 2 **Average and standard deviation of filterability G_{max} [g] ($n = 10$) using cellulose nitrate (CN), polyamid (PA) and polyethersulphone (PES) membranes. legend: ■ control beer, ■ beer + fatty acids, ■ beer + MCFA ethyl esters**

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.

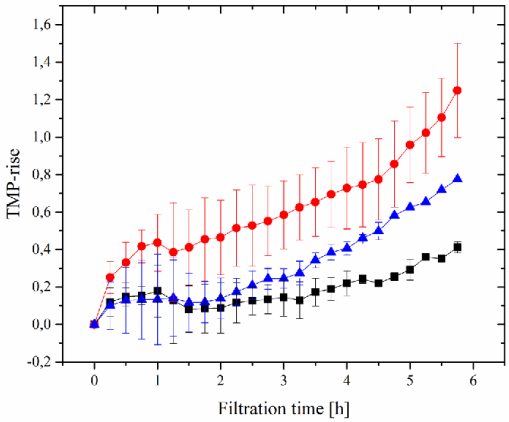

Fig. 3 **Average and standard deviation ($n = 3$) of trans-membrane pressure (TMP) rise over filtration time. legend: ■ control beer, ▲ beer + MCFA ethyl esters, ● beer+ fatty acids**

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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

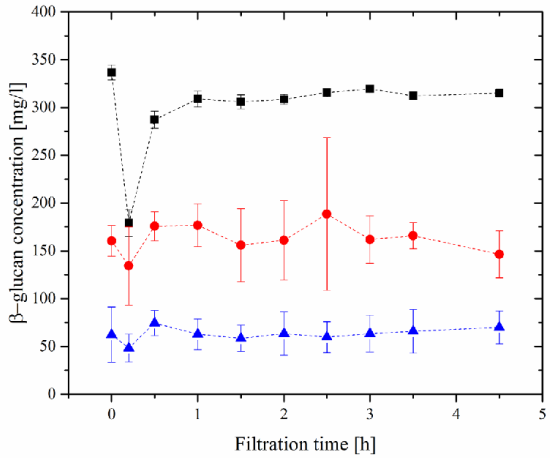

Fig. 4 β-Glucan concentration (n = 3 × 4) determined using fluorometric assay, legend: ■ control beer, ▲ beer + MCFA ethyl esters, ● beer+ fatty acids

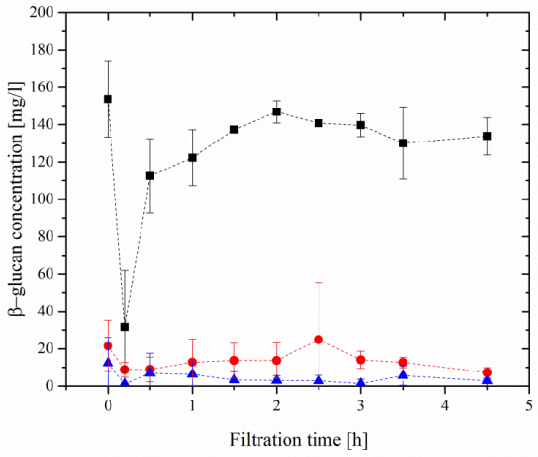

Fig. 5 β-Glucan concentration (n = 3 × 4) in the course of CFMF determined using colorimetric assay, legend: ■ control beer, ▲ beer + MCFA ethyl esters, ● beer+ fatty acids

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

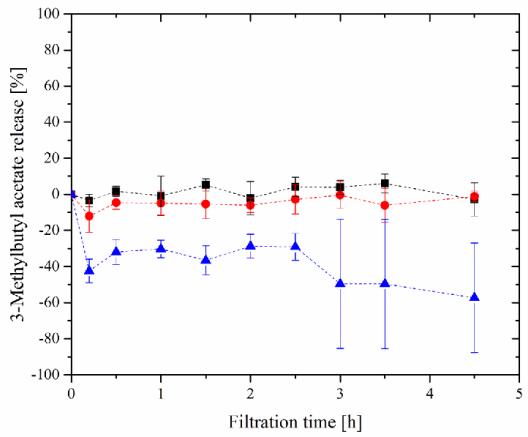

Fig. 6 **Aroma release [%] of 3-Methylbutyl acetate ($n = 3$) in the course of CFMF determined by GC-FID, legend: ■ control beer, ▲ beer + MCFA ethyl esters, ● beer+ fatty acids**

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.

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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4 Discussion

Filterability of beer is an important factor in relation to the stability and durability of the product. During the beer filtration process, these properties can be influenced by a steeper increase in filter pressure and variations in turbidity, mainly affected by the composition of the unfiltered beer. In particular, an examination of total polymer concentrations like proteins and polysaccharides should provide an indication of filter performance and the turbidity of beer. However, these investigations yielded only limited success due to the large number of beer ingredients. Specific issues regarding interactions among beer ingredients or with filter media were only partially considered. However, this is essential to gain precise knowledge of the technological process design.

The present work provides a fundamental contribution to investigating the impact of β-glucans on filtration performance during DE precoat and membrane filtration of beer. Besides the investigation of total polysaccharide content, the effect of β-glucan molar mass, geometry and origin (yeast cell wall or barley) was examined. The β-glucan content of beer could be measured using different methods based on enzymatic breakdown, acid hydrolysis or staining using specific dyes. Apart from the total β-glucan content, certain molar mass ranges of β-glucan can be considered in connection to membrane or DE filtration performance [115,116]. No correlation to either membrane or DE filtration was found for total β-glucan concentration (see chapter 3.2). Thus, information of applied quantification methods (enzymatic method or staining with Calcofluor White or Congo red) regarding beer β-glucan content have great variations. In order to obtain detailed information on filterability, the differentiation of filtration-inhibiting molar mass ranges of β-glucans is essential. Besides high molar mass barley β-glucans (> 1.0×10^5 g/mol), a high β-glucan gel content had a negative impact on membrane filtration [95,104,106]. This is not surprising, since high molar mass β-glucans are known for their increased agglomeration potential, which could lead to a stronger clogging of membrane pores (compare chapter 2.3, page 18–19) [67]. Furthermore, high concentrations of low molar mass β-glucans (1.0×10^4–1.0×10^5 g/mol) were identified as having a negative influence on membrane filtration performance. To quantify these molar mass fractions, a fluorimetric assay using

Calcofluor white staining was most suitable [117]. However, the impact of β-glucan gel was only investigated considering total gel concentration but not by the degree of agglomeration or the particle size of these agglomerates [110].

Comparable results could be found for DE filtration, where increased gel content decreased filter performance [95]. Investigations regarding the molar mass of β-glucans could not be found in literature (compare chapter 3.2). Furthermore, none of the evaluated β-glucan quantification methods provided consistent information for DE filtration [117]. Nevertheless, a connection between increasing viscosity and decreased DE precoat filter performance was described by several authors [118,119]. Since the viscosity measurements represent not only the behaviour of β-glucans but all the components dissolved in the beer, DE precoat filtration may not only be influenced by the β-glucan composition of beer [95].

However, the considered β-glucan assays only allow a statement about the composition of cereal β-glucans in beer. Kreisz [6] showed that polysaccharides derived from yeast had a great effect on turbidity and filterability. Here, varying effects could be found in membrane and DE filtration (see chapter 3.3). Besides a decrease in the filtrate flow of nearly 90% during membrane filtration, complete membrane clogging could be observed after a few seconds' filtration time (see chapter 3.3, page 45, Fig. 7). In contrast, DE filtration performance decreased by 20% with a constant volume flow until the end of filtration (see chapter 3.3, page 45, Fig. 7). This suggests that the inclusion of yeast β-glucan molecules in the filter cake allowed the continuation of filtration, whereas deposition in or on polymer membranes resulted in a total clogging of pores. Observed results confirm knowledge from literature and shows the distinct differences between sieve and cake filtration.

Further ingredients which can be obtained in beer via yeast cell lysis besides yeast β-glucans are different volatiles. In this context, Eagle et al. [86] found no influence of ethyl acetate on membrane filtration, which could also be confirmed by filtration trials shown before (see chapter 3.3, page 44, Fig. 4). In contrast, a decline of MCFA ethyl ester was observed during DE and membrane filtrations. Regardless of the β-glucan concentration, the MCFA ethyl ester decreased during DE precoat filtration by up to 90%. Furthermore, a stronger decline in ester content was determined with increasing chain length of the fatty acid residues (see chapter 3.3, page 45, Fig. 6). However, the

addition of ethyl esters to model beer solutions not only resulted in the retention of volatiles during DE filtration, but also in a decreasing filtrate flow of up to 40%. Furthermore, a decrease in filter performance could be measured with increasing barley β-glucan concentration. This is accompanied by the knowledge of literature [118]. The addition of volatiles to β-glucan-containing model beers resulted in a stronger impact on filter performance (up to 65%) and retention of MCFA ethyl esters (over 90%) during membrane filtration (see chapter 3.3, page 42/43). Independent of the β-glucan concentration of the unfiltered model beer, the membrane material used affected concentrations of ethyl octanoate (−58%), ethyl decanoate (−87%) and ethyl dodecanoate (−94%). Comparable to DE filtration, a drop in filter performance with rising β-glucan content was found in membrane filtration trials (see chapter 3.3, page 44, Tab. 1). However, degradation was significantly higher compared to DE filtration.

Responsible for this combined effect of barley β-glucan and volatiles was an agglomeration of polysaccharide molecules. An increase in molar mass distribution due to the addition of volatiles could be observed in β-glucan model beer solutions (see chapter 3.4, page 53, Fig. 2). However, this effect was dependent on volatile molecular structure and chain length of fatty acid or alcohol residues. In this case, clear differences were found in spite of the same log K_{OW} value of the studied isomers (dodecanoic acid, octyl butyrate, ethyl decanoate and decyl acetate, compare chapter 3.4, page 52, Tab. 1). Besides an increase in molar mass, viscosity and β-glucan gel content of the model beer increased due to the addition of volatiles. Aggregation of β-glucans and thus gel building could be determined by investigating radii of gyration and hydrodynamic radii (aggregation number: $x^2_{r,control}$ =1.9, $x^2_{r,decyl\ acetate}$ = 2.2, compare page 53, Tab. 5). An increased association of β-glucan molecules could be found depending on the chain length of the fatty acid or alcohol residue of the investigated volatiles. This enhanced agglomeration occurs due to a degradation in solubility of the polysaccharides in the corresponding solvents. Similar reactions are also possible in beer due to the specific composition of β-glucans and volatiles from yeast fermentation. In this context, different authors assumed a decreasing filterability during DE precoat filtration due to a dosage of cold break to beer, mainly consisting of high amounts of β-glucans [94,95,97,120,121]. The addition of cold break resulted furthermore in a drastic increase in beer viscosity [94]. The impact of volatiles on cold break composition was not determined by the authors. In contrast, decreased

filterability with cold break dosage to beer could not be observed during membrane filtration [95].

To examine the combined effect of volatiles and β-glucans on membrane filtration and filter clogging, locally-resolved image analysis using CLSM was performed (see Figure 4-1).

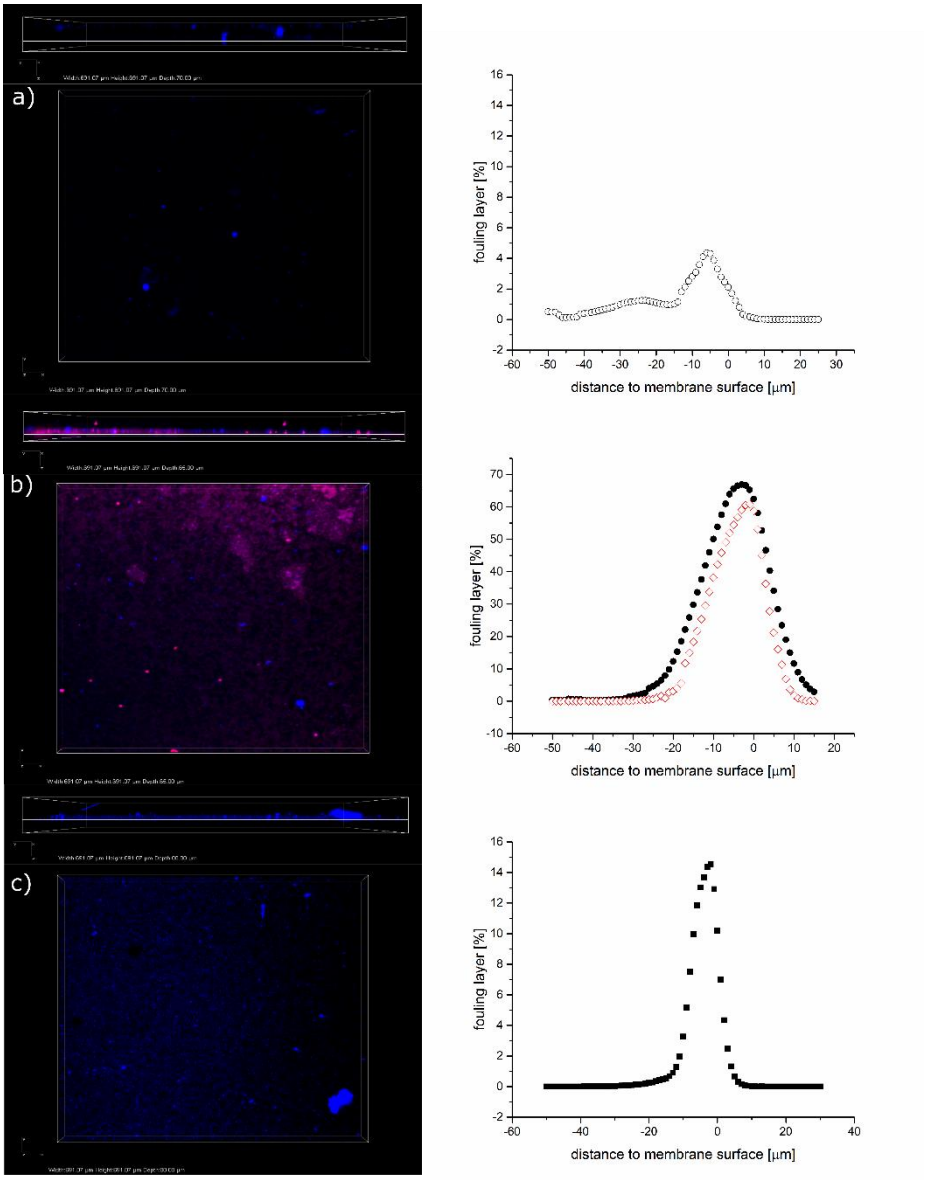

Figure 4-1: Locally-resolved image analysis using CLSM (z- and x-axis view) and graphical analysis of fouling layers on PES membranes (0.45 µm pore size): a) 50 mg/l barley β-glucan (medium viscosity) (○, blue), b) 50 mg/l barley β-glucan (●, blue) and 100 mg/l decyl acetate (◇, red), c) 50 mg/l yeast β-glucan (■, blue), CLSM method: staining using Calcofluor White 1:10 diluted in Tris-HCl buffer (pH 8) and Nile red diluted in ethanol (1:100,000); detection: 20-fold magnification, Argon-ion laser (488 nm wavelength): HV: 100, offset: -60 and red-diode laser (635 nm wavelength): HV: 60, offset: -60 [122].

For the investigation of layer formation on PES membranes, model beer solutions consisting of 50 mg/l barley β-glucans in 5% (w/w) ethanolic solution were filtered. It was found that pure barley β-glucan solutions had only a low fouling (maximum 5%) on the membrane surface, with only a few larger particles. This is consistent with the results from chapter 3.3, where an impact of β-glucan was first observed at a concentration of 200 mg/l. In contrast, model beer containing 50 mg/l β-glucan and 100 mg/l ethyl decanoate had a higher layer on the membrane surface. Fouling of β-glucans reached nearly 70% on the membrane surface, while ethyl decanoate covered 60% of the membrane surface. Furthermore, a broader coverage, also inside the membrane, could be determined in this sample. One conspicuous difference was the detection of larger polysaccharide particles on the membrane in spite of the same concentration of β-glucan. This is consistent with the findings of chapter 3.4, which reinforces β-glucan retention due to a degradation in solubility and polysaccharide agglomeration. Furthermore, deposits were mainly found on PES membrane surfaces.

In addition to barley β-glucans, yeast β-glucan (50 mg/l) models were investigated and a fouling of maximum 15% on the membrane surface was found. A thin layer and some bigger particles could be detected on the entire membrane surface. To expose retention and clogging mechanisms of investigated polysaccharides, the molar mass distribution of used β-glucan standards was investigated with an asymmetric field-flow fractionation (measurement principle described in chapter 3.4). It could be observed that yeast β-glucan had a lower medium molar mass (M_W = 2.5 ± 0.2×10^5 g/mol, n = 2) and lower dispersity (M_W/M_n = 1.3, n = 2) in comparison to barley β-glucan (M_W = 2.8 ± 0.1×10^6 g/mol, M_W/M_n = 1.6, n = 3). Nevertheless, higher radius of gyration ($r_{rms,\ yeast}$ = 120.0 ± 24 nm, n = 2; $r_{rms,\ barley}$ = 85.8 ± 29.5 nm) and comparable hydrodynamic radius ($r_{h\ yeast}$ = 103.5 ± 51.3 nm, n = 2; $r_{h,\ barley}$ = 101.0 ± 0.7 nm) could be found for yeast β-glucan, resulting in a more linear structure of this polymer (υ_{yeast} = 0.62), whereas barley β-glucan had a random coiled structure (υ_{barley} = 0.54). This is a clear indication of the influence of molecular geometry and expansion on its filtration properties during membrane separation processes. The described impact on membrane clogging and filter performance was investigated in lager beer. Figure 4-2 shows the filter performance of model beer samples as a function of β-glucan concentration and the addition of ethyl decanoate and decyl acetate. The two volatiles

which had the greatest influence on β-glucan agglomeration during the molar mass studies proposed a substantial reduction in filter performance analysing PES membranes. The investigation of membrane clogging showed a significant increase in β-glucan content on the membrane surface by the addition of volatiles. Above all, the addition of the MCFA ethyl ester ethyl decanoate resulted in β-glucan-induced membrane clogging greater than 70% (see Figure 4-2b). Furthermore, the investigation of membrane clogging after filtration of lager beer samples was undertaken to compare previously examined findings. In addition to filter performances of less than 2.5 g/(min×cm^2×bar), fouling layers greater than 60% could be measured. Moreover, membrane clogging of β-glucans in beer samples was comparable to model beer samples containing volatiles.

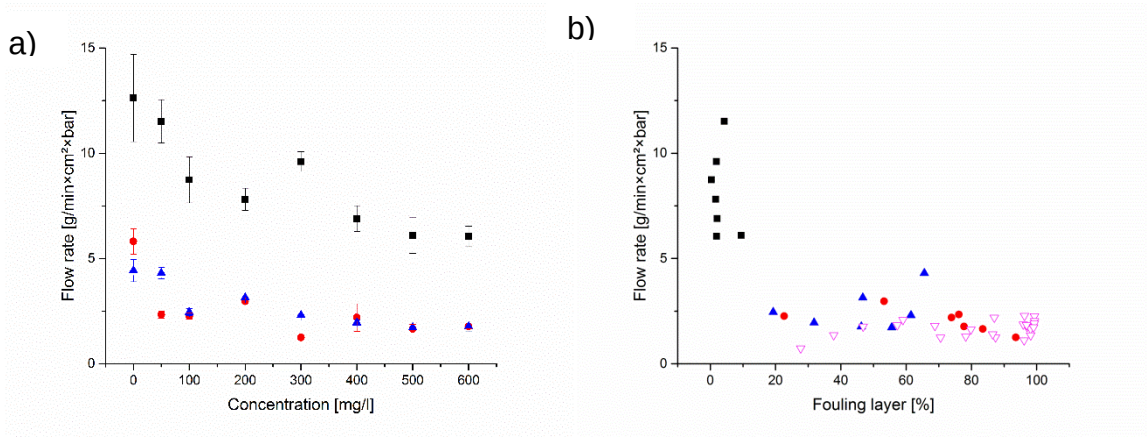

Figure 4-2: Filtrate flow (n = 3) of beer model solutions (5% (w/w) ethanol) in dependence on their β-glucan concentration using PES membranes (A, measurement principle described on page 41) and fouling layer degree (n = 12, measurement principle described in Figure 4-1) of the β-glucans in dependence on the flow rate of these beer model solutions (n = 3, control barley β-glucan sample (■), barley β-glucan + 100 mg/l ethyl decanoate (●), barley β-glucan + 100 mg/l decyl acetate(▲)) and 26 lager beer samples (▽) on PES membrane (B).

Comparable results were described in literature, where an influence of polysaccharide geometry on retention could be detected [51]. Furthermore, the impact of gel formation on membrane pore clogging was described by Agbangla et al. [48]. This described agglomeration (“gelified accumulation”) of polymers is enhanced for beer β-glucans due to the decline in solubility by the addition of volatiles (see chapter 3.3 and 3.4). β-Glucan aggregation is known from literature due to high molar mass fractions (> 1.0×10^5 g/mol) and high concentrations of β-glucan, which is further enhanced due to a change in solubility [67].

According to Figure 2-5, filter clogging can also occur in the presence of particles smaller than membrane pore size. This pore bridging, a special type of standard blocking, can lead to “bridges” above the membrane pore and increase the deposition of particles on the membrane surface [48,123]. Because of the layer formation shown in Figure 4-1b, comparable mechanisms might be possible. Referring to Equation 2-6, the internal resistance ($R_{h,1}$) as well as cake resistance ($R_{h,2}$) increases, which results in a reduction of filter performance. It could be demonstrated that β-glucan composition has a great influence on membrane clogging during beer filtration. With the help of locally-resolved image analysis of the membrane clogging, it was possible to detect above all cake layer formation as well as intermediate blocking and less in-pore blocking caused by β-glucans. Furthermore, an impact of the observed interactions on filter clogging in dependence on the used membrane material could be determined (see chapter 3.5, page 60, Fig. 2). Most hydrophilic membranes manufactured from cellulose nitrate had nearly no decline in filterability due to filtration of beer and beer with dosage of MCFA ethyl esters, but with dosage of longer chain fatty acids (C_{18}–C_{22}). In comparison, polyethersulphone and polyamide membranes had a decrease in filterability due to the addition of hydrophobic substances to beer. Filterability was associated with chain length of the fatty acid (residues) of investigated agents, which is consistent with the findings of the previous chapters. During crossflow filtration trials, the faster increase in pressure due to the addition of flavouring substances to beer was examined. This increase in pressure had an additional negative effect on filter service life.

Based on the analytical data, it can be established that membrane filtration is more strongly affected by described β-glucan agglomeration due to the presence of volatile than DE precoat filtration. This could be mainly observed due to the layer formation on the membrane surface because of interactions of beer ingredients with the membrane material. During DE filtration, the inclusion of inhibitory substances in filter cake resulted in a continuation of filtration processes. Beer that is difficult to filter can be counteracted by adjustments to the amount of filter aid and to the porosity of the filter cake. The type of beer ingredients that affect DE filtration is less important than their effect on liquid viscosity. This could be illustrated with both β-glucan-volatile and yeast β-glucan filtration, as these ingredients have a large impact on beer viscosity. Besides an inclusion, adsorption and sieve effects could be observed during DE filtration [124],

which can be enhanced because of increased beer viscosity due to a reduction in flow velocity through the cake. In contrast, membrane filtration observed a strong effect of molecule geometry on filterability and filter clogging. In addition, an influence on hydrophilic qualities of membranes in connection to sample composition could be found. Although comparable substance groups are involved in filter clogging and the degradation of both filtration processes, different retention mechanisms could be identified during membrane and DE precoat filtration. In connection to beer composition, not only polymer substances from malt but also yeast metabolism products must be considered in beer filterability. These autolysis products complicate the predictability of filtration processes in upstream process steps of brewing.

Since one of the main filtration-inhibiting substance groups in membrane filtration could be identified, the next step must be process optimization with regard to beer filtration and beer production to increase filter service lifetime. A modification of the membrane material composition can cause a change in the deposition of β-glucans and volatiles as well as a simultaneous increase in filterability. The first findings on this topic could be achieved within this work (see chapter 3.5). Furthermore, cake formation on membrane surfaces can be prevented by means of modifications to crossflow filtration process technology like circulation speeds. For an additional improvement of membrane filtration, irreversible fouling caused by β-glucan gel layers can be effectively removed by chemical cleaning [41]. An adaptation of cleaning processes as well as the detailed analysis of beer membrane fouling are essential to ensure a successful filtration process and the desired beer stability.

In addition to filter process technology, beer production provides a great potential for improving filterability. First, careful selection of raw materials allows the usage of malts with low concentrations of β-glucans. Moreover, the degradation of these polysaccharides during mashing can be attempted. To prevent interactions of β-glucans with MCFA ethyl esters, a high yeast quality regarding vitality and viability must be considered. This is necessary because longer chain MCFA ethyl esters (e.g. ethyl decanoate) are mainly extracted from the cell in the case of lysis, which had a larger negative impact on filterability. In addition, yeast β-glucans can enter into the fermentation medium, which greatly impairs filterability. Thus, not only the brewing process but also fermentation should be monitored more closely to obtain a good level of filterability and beer quality.

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6 Appendix

6.1 Non-reviewed papers

1. Kupetz, M., Zarnkow, M., Becker, T. (2013). *„Schnelltests zur Aufklärung von Filtrationsproblemen."* Jahrbuch der Weihenstephaner 83(1): 26-29
2. Kupetz, M., Herkersdorf, M., Zarnkow, M., Becker, T. (2014). *„Schnelltest zur Identifizierung filtrationshemmender Stoffgruppen."* Brauwelt 29: 866-869
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4. Kupetz, M., Krauß, M., Sacher, B.; Becker, T. (2015). *„Photometrischer Jodwert- für die Filtrierbarkeit aussagekräftig?“*, Brauwelt 14/15:396–398
5. Kupetz, M., Herkersdorf, M., Zarnkow, M., Becker, T. (2015). „Método Rápido para la Identificación de Grupos de Substancias que Frenan la Filtración.” Brauwelt en Español 19: 63–67
6. Kupetz, M., Pohler, D., Fischer, S., Becker, T. (2016) „*Das volle Spektrum der β-Glucane.“.* Brauwelt, 31/32: 893-897
7. Kupetz, M., Herkersdorf, M., Zarnkow, M., Becker, T. (2016) *“Rapid test for identification of filtration-inhibiting substance groups”*. Brauwelt international, 34: 322–325
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10. Kupetz, M., Krauß, M., Sacher, B.; Becker, T. (2017). „Valor del iodo fotométrico: ¿ de valor informativo para la filtrabilidad?“. Brauwelt en Espaoňl 21: 72–74

6.2 Oral presentations with first authorship

11. Kupetz, M., Qian, F., Becker, T. (2012) *„Überprüfung eines neuartigen Messverfahrens mittels Ladungstitration zur Überprüfung der Hefevitalität“*., 45. Technologisches Seminar, Freising, Germany
12. Kupetz, M., Zarnkow, M., Becker, T. (2013). „*Kritische Betrachtung der Filtrationstests im Labormaßstab – Auswertungsmöglichkeiten und Ergebnisse“,* 46. Technologisches Seminar, Freising, Germany, 19.02.2013
13. Kupetz, M., Zarnkow, M., Becker, T. (2013). *„Investigation of the filterability of beer using different layer formation in the static and dynamic membrane filtration.”*, Filtech, Wiesbaden, Germany, 23.10.2013
14. Kupetz, M., Zarnkow, M., Becker, T. (2013). *„Validity of the Laboratory Filtration as a Prediction of the Filterability of Beer."* Proceedings of the 34th Congress European Brewing Convention Luxembourg
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16. Kupetz, M., Zarnkow, M., Becker, T. (2014) *„Interactions between β-glucan and flavouring compounds in relation to filterability of beer”, 11th Trends in Brewing, Gent, 16.04.2014*
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6.3 Poster presentations with first authorship

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6.4 Curriculum Vitae

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10/2006 – 10/2011	Diploma Ingenieur of brewing and beverage technology at the Technical University of Munich Diploma thesis: "Verification of a new measuring method using charge titration to evaluate the yeast vitality"
2009 – 2011	Student research assistant at TUM Chair BGT (Working group: Process automation)
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