



Influence of dextrins and β -glucans on palate fullness and mouthfeel of beer

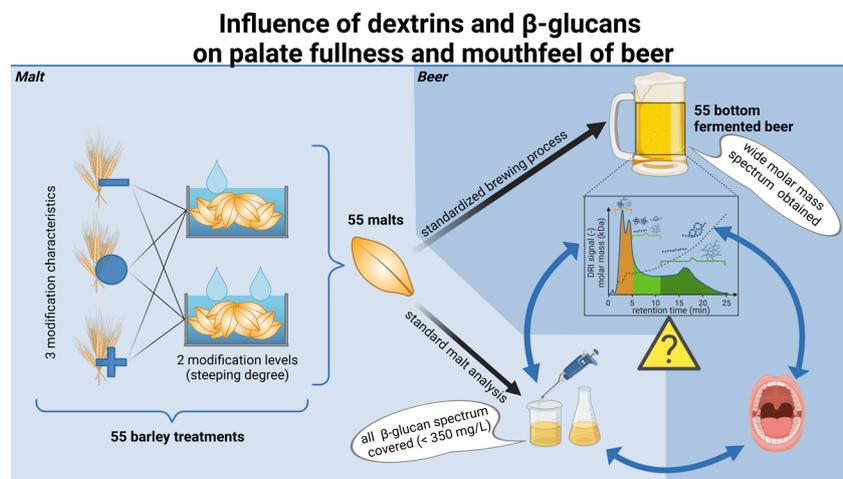
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Abstract

Palate fullness (PF) and mouthfeel are important sensory attributes influencing beer quality. The molar mass of starch (dextrins) and non-starch (β -glucans) polysaccharides may influence *PF* (pleasant) or mouthfeel (*sliminess*, unpleasant), respectively. Therefore, this research aims to generate beer with wide physico-chemical responses based on various raw material characteristics to study its relation to *PF* and *mouthfeel*. To accomplish this, ten barley varieties (two harvest locations and years) were classified into three groups based on their modification characteristics. To intensify response variation, barley was malted at two modification levels (parameter steeping degree), generating 55 independent malts used to brew the same number of standardized bottom fermented beers. A trained sensory panel evaluated *PF* (*intensity* and *quality*) and *mouthfeel* (e.g., *slimy*) descriptors. Additionally, beers were fractionated by asymmetrical flow field-flow fractionation (AF4) in three different fractions and their molar masses were determined. The average molar mass of big size (> 10 nm) dextrins and β -glucans AF4 fraction increased analogously to barley modification characteristics. For sensory data evaluation, only beer samples brewed with malts inside the recommended brewing specifications were considered (β -glucan content in malt < 350 mg/L, ISO 65 °C). *PF quality* was lower on samples with β -glucan content < 100 mg/L. This group also contained fewer dextrins (attenuation $> 87.4 \pm 1.5\%$), suggesting that the balance of β -glucans and dextrins is important for *PF quality*. This work indicates that molar mass of starch and non-starch polysaccharides, affected by barley variety and its modification level, influences sensory perception, and hence, beer quality.

Graphical Abstract



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Extended author information available on the last page of the article

Keywords Beer · Barley malt · β -Glucans · Dextrins · Molar mass · Palate fullness

Introduction

Barley is the most used grain for brewing in the world, usually as barley malt. Malt quality is of great consequence in beer production and thus has a substantial impact on the overall nature of the finished beer. Many studies confirm the negative effects of inadequate malt quality [8, 9, 21, 36, 46]. Some parameters heavily influenced by malt quality are individual production steps (e.g., lautering, fermentation, and filtration) and attributes central to beer character (e.g., flavor, color, foam, and stability) [1]. A few sources have focused on the influence of barley malt modification and beer sensory quality [23, 43]; however, the connection among malt modification, physico-chemical characteristics (standard malt analysis, e.g., limit attenuation, β -glucans), and sensory perception is not fully understood.

The sensory characteristics, such as *palate fullness* (PF) and *mouthfeel*, are relevant to improve consumer's acceptance of a beverage. On beer, the factors influencing sensory perception of *palate fullness* (PF) and *mouthfeel* were studied by Langstaff during the early 1990s [27]. This research resulted in an adaptation of the ASBC Beer Flavor Wheel which thoroughly describes beer *mouthfeel* [26]. The term "fullness," weight resistance to flow related to the viscosity and density of the sample, in the wheel is indicated as part of *mouthfeel* descriptors. 50 g/L of dextrin concentration was detected to induce a viscosity change in carbonated beer [40] and, theoretically, influence the PF according to Langstaff's definition. Rübsum et al. expanded the definition of (*palate*) *fullness* by showing that this attribute is dependent on the non-volatile beer matrix composition with focus on molar distribution by spiking maltodextrins with different sizes into beer [43]. Interestingly, lower concentrations were needed (5–20 g/L) to modulate PF intensity compared to the previously mentioned work. The concentration difference between these investigations suggests that viscosity changes cannot be the sole determinant of sensory response changes in beer.

Different non-volatile substance groups and their molar masses, as well as total macromolecular concentration, also affect the perception of PF and *mouthfeel* [21]. Accordingly, Krebs et al. showed that besides molar mass, the individual substance group influences the sensory characteristics of the product. Non-alcoholic beer spiked with dextrins was considered as pleasant (positive effect) while adding β -glucans increased the *slimy mouthfeel* perception (negative effect) [23]. To sum up, PF and *mouthfeel* are dependent not only on molar mass but also on the substance group (β -glucans or

dextrins), which in turn could have a positive or negatively effect on consumer acceptance.

The final composition of the non-volatile beer matrix is greatly influenced by raw material (malt specifications). The malting process is divided into three parts: steeping, germination, and kilning. The malt quality is mainly altered by three parameters: germination time, steeping degree, and temperature. During malting by means of a controlled germination of grain, specific enzymes are generated that are necessary in the brewing process. Furthermore, the aims of malting are a partial but adequate degradation of high molecular compounds such as proteins and hemicelluloses (arabinoxylans and β -glucans) in the cell walls of the grains' endosperm. Even though up to 90% of β -glucan can be degraded during malting, there is no common consent on how malting affects arabinoxylan concentration decrease; nevertheless, degradation does occur [13]. In turn, proper degradation achieves a sufficient malt processability during brewing with respect to high extract yield, proper fermentation, and sufficient filtration processes [33].

During malting and mashing, protein materials are degraded into amino acids and peptides necessary for fermentation (e.g., yeast metabolism) [12]. Protein is majorly solubilized during the malting process [19]. The level of protein modification is assessed in the brewing industry by the soluble nitrogen [46]. Regarding protein molar mass changes by malting, higher soluble nitrogen content (in consequence higher Kolbach indices at the same protein content), thus higher protein modification, in wheat malt led to concentration decrease of high molar mass proteins (36.6–70.8 kDa), while the smaller fraction increased (14.9–35.0 kDa) [29].

The analytical parameters of barley malt analysis describe the three primary modification processes that occur in the kernel during the malting process: cytolysis (cell wall degradation), proteolysis (protein degradation), and amyolysis (starch degradation) [1]. Specifications (analytical guide values) define the quality of malt required for effortless processing. Thus, they have become the standards used by malt producers and other processing companies to guarantee processability in the brewery [1, 9]: amyolytic criteria (malt parameter) are composed of extract, final attenuation, and the amyolytic enzymes α - and β -amylase; proteolytic criteria are composed of crude protein content, soluble nitrogen (SN), Kolbach index (calculated value), and free amino nitrogen (FAN); and cytolytic criteria contain friability, viscosity, and β -glucan content [1].

High-quality barley malt was achieved as a result of breeding improvements with the focus on cytolytic

degradation (low β -glucan content, low viscosity, and high friability) over the past 50 years [8]. When these varieties are additionally balanced in their modification behavior (proteolysis and cytolysis), the usage of high modification varieties, as previously described, is interesting for the brewing industry due to economic (reduction in malting cost) and environmental (less water footprint) reasons. Consequently, due to the considerably advanced proteolytic and cytolytic modification of barley grain accomplished in the malthouse, brewers can focus their efforts on degrading the starch in the mash vessel [9, 34].

Amylolysis and cytolysis mean not only the concentration but also how the molar mass of starch (dextrins) and non-starch polysaccharides (β -glucans) is affected during malting and brewing processes. During the malting process (germination), degradation of β -glucans [31] and arabinoxylans [5, 11, 25] generate smaller molar masses. In general, an increase in barley malt modification leads to a decrease of cell wall polysaccharides [21]. Longer germination time and higher temperatures promote cytolysis and increase the amount of low molar mass arabinoxylans [15] and β -glucans [14]. Regarding the brewing process, different mashing temperatures generate a diverse molar mass response due to different enzymatic activities (the mash pH and the temperature of the individual mash rests) [42].

Analytically for cereal-based beverages (including beer), fractionation and molar mass determination may be performed by size exclusion chromatography (SEC) and asymmetric flow field-flow fractionation (AF4) coupled to multi-angle light scattering (MALS) and concentration detectors (differential refractive index, DRI) [44]. Both techniques fractionate molecules according to their hydrodynamic size, but the lack of a stationary phase in the AF4 makes it a more versatile technique [39]. Literature examples of molar mass characterization on brewing systems include diverse matrices such as barley malt [31], beer [47], and spent grains [49].

To sum up, former studies indicate that the type of substance (starch or non-starch polysaccharides – dextrins or β -glucans) and the molar mass distribution in the final beer influence the sensory properties of cereal-based beverages. Apart from polymer precipitation during the brewing process (boiling, trub formation) and the AF4 10 kDa membrane; assuming a standardized brewing (mashing) process as usual practice, the macromolecular profile remains relatively unmodified during fermentation since yeast cannot metabolize high molar mass substances. Thus, the macromolecules are solely depolymerized during the malting and mashing process by the malt's intrinsic enzymes. This confirms a molar mass dependency on raw material characteristics (malt parameters reflecting degree of modification) for the product quality. However, there is a knowledge gap regarding the role of molar mass variation in respect to *PF* and *mouthfeel* of bottom fermented beer focusing on starch

and non-starch polysaccharides (dominating substance group or synergistic effects) when using barley malt with different modification levels (based on the β -glucan substance) and varieties with different modification characteristics.

Ten malting barley varieties were chosen and previously classified as low, moderate/medium, and high according to their modification characteristics [10]. Samples were malted at two modification levels (low and high modified using steeping degree as adapted malting parameter based on maltsters' expert knowledge) to generate different modified malt samples, resulting in a wide spectrum of molar mass responses of the standardized brewed bottom fermented beer samples.

In the first part of this study the standard malt analysis (malt parameters, SMA) and molar mass responses of their resultant beers were compared depending on modification levels of the final malt under consideration of the malting barley modification characteristics (from which the malt was produced). The beer molar mass was classified in three substance group fractions [20]: 1. proteins (22–32.7 kDa); 2. protein–polyphenol complexes (P-PC) and low non-starch polysaccharides (42.7–65.9 kDa); and 3. high non-starch polysaccharides ($2.63\text{--}25.07\cdot 10^3$ kDa). Due to the possible presence of dextrins in all fractions, the low and high molar mass (non-)starch polysaccharides (N-SP) present in fractions 2 and 3 will be referred as LN-SP and HN-SP, respectively.

Despite several parameters change during malting, in the second part involving sensory analysis results, special focus was only given to samples that comply with the β -glucan specification (< 350 mg/L in malt, ISO 65 °C) [1], thus elucidating how the molar mass responses influence the sensory perception of bottom fermented beers from the practical brewing scenario. Finally, the relationship among analytical data of molar mass, concentration, and sensory was investigated to differentiate how molar mass fractions of starch and non-starch polysaccharides could positively or negatively affect the *PF* and *mouthfeel* of beer and to what extent they can be controlled by malt modification levels and variety characteristics.

Materials and methods

Malting procedure and standard malt analysis

Malting barley samples from ten different varieties with differing malting modification characteristics (modification behavior during the malting procedure) were used on this study. The samples were derived from two different harvest years (2017 and 2018) and locations (A and B). This resulted in 55 different malt samples as shown in Table 1. Two modification levels were achieved by defined variations

Table 1 Ten malting barley varieties classified by cytolytic modification characteristics and adjusted steeping degrees used during the malting procedure to achieve two different modification levels ($n = 55$). LSD, low steeping degree; HSD, high steeping degree; n.a. not available

Harvest year		2017		2018			
		Location A		Location A		Location B	
Variety	Classification	LSD (%)	HSD (%)	LSD (%)	HSD (%)	LSD (%)	HSD (%)
A	Low	41	44	40	43	n.a	43
B	Low	41	44	41	44	40	42
C	Low	41	44	40	43	40	43
D	Low	n.a	n.a	43	46	42	44
E	Moderate	42	45	42	46	40	42
F	Moderate	n.a	n.a	40	43	40	42
G	Moderate	41	44	40	43	39	41
H	High	41	43	40	43	39	42
I	High	39	41	39	42	39	42
J	High	39	41	39	42	40	42

of malting parameter steeping degree as a tool to modify the grains, resulting in a wide range of molar mass responses in the malt and in consequence in the corresponding standardized brewed bottom fermented beer. The steeping degree level for each sample was set based on maltsters' expert knowledge based on variety trials.

The malting barley varieties were categorized by their modification characteristics as low, moderate, and high according to their modification behavior during the malting process based on breeders' expertise and results of the German malting barley variety evaluation program, "Berliner Programm" German Brewing Barley Association [2]. A micro-malting system was used to produce barley malt (1 kg scale) and were malted as standard according to MEBAK analogous to "German malting barley variety evaluation program procedure" R-110.00.008 [2016–03] [18], malting with only changes of the steeping degree as previously stated (Table 1). SMA were determined according to MEBAK [18]: Amylolytic (extract, > 81% R-205.01.080 [2016–03]; final attenuation, > 81% R-205.17.080 [2016–03]), proteolytic (crude protein, 9–11%, R-200.20.030 [2016–03]; soluble nitrogen, 570–670 mg/100 g DM, R-205.11.030 [2016–03]; FAN, 100–140 mg/100 g DM, R-205.14.111 [2016–03]), and cytolytic (friability, > 82%, R-200.14.011 [2016–03]; viscosity, < 1.60 mPa s, R-205.10.282 [2016–03]; β -glucans, < 350 mg/L, R-205.15.174 [2016–03]) parameters. The specification values in parenthesis of the previously mentioned parameters are for malt following isothermal mash at 65 °C (ISO 65 °C) [1].

Brewing process

The malting setup leads to 55 independent malt samples used to brew the same number of standardized bottom fermented beers. Lager beers (original gravity 11 wt%, 5% ethanol by volume, 25 bitter units) were brewed in an 8 L

automated small-scale brewery (Joh. Albrecht Brautechnik JBT GmbH, Germany). 1.5 kg of malt was milled in a 2-roll dry malt mill (MIAG, Germany). A standardized infusion mashing procedure was carried out, the starting temperature was set at 62 °C for 30 min followed by a temperature increase rate of 1 °C per minute until reaching 72 °C for 30 min. Afterward, the mashing temperature was increased to 76 °C and was held for 10 min before lautering. The wort was boiled for 1 h and the hop extract added at the beginning of wort boiling was calculated to achieve 25 bitter units (CO₂ extract Hallertuer Herkules, HVG, Germany). After a whirlpool rest of 10 min, the worts were cooled. Yeast (Saflager W-34/70 by Fermentis, France, rehydrated and allowed to revive in first wort) was added. The worts were fermented for 5 days at 12 °C until their extract value dropped below 3.5% w/w. Green beer was matured at 16 °C until it reached a total diacetyl value below 0.1 mg/L (analyzed according to MEBAK 2.21.5.1 [18]). The beers were stored at 0 °C for four weeks and filtered (three filter layers, K150, Pall Corporation, Germany). All sample beers were filled in 0.33 L brown longneck bottles under CO₂ and stored at 4 °C.

Molar mass determination by asymmetrical flow field-flow fractionation

Molar mass of all the beer samples was determined by AF4 coupled to MALS and DRI. The method used was formulated by Krebs et al. 2017. Briefly, fractionation takes place in a separation channel with a 350 μ m spacer. A 10 kDa regenerated cellulose ultrafiltration membrane was placed at the bottom of the channel. The eluent buffer was composed of 50 mM sodium nitrate and 0.025% NaN₃. A 0.1 μ m filter was placed between the pump and the autosampler to avoid big particles entering into the device.

A quaternary pump (Agilent 1100 series, Agilent Technologies, Germany) fed the solvent into the system. Flows

in the separation channel were controlled by an Eclipse 3+ instrument (Wyatt Technology Europe, Germany). 100 μL of beer was injected to the system via an autosampler (Agilent 1100 series, Agilent Technologies, Germany). Each sample was measured in duplicate. Before injection, the beer was degassed for 5 min by sonication and filtered through a 0.45 μm polyester syringe filter (Chromafil, Macherey–Nagel, Germany). The sample was focused for 8 min with a focus flow of 4 mL/min. During elution, the initial crossflow was fixed at the same rate as the focus flow. The detector flow was set at 1 mL/min. After 5 min, the crossflow was decreased linearly to 0.2 mL/min over 10 min to later be further reduced to 0 mL/min during the next 10 min. The channel was rinsed for 15 min with the injection valve re-opened for the first 5 min before the next injection. After fractionation, the sample was passed through UV (Agilent 1200 series, Agilent Technologies, Germany), MALS (DAWN HELEOS II, Wyatt Technology Europe, Germany), and DRI (Agilent series 1260 RID VIS-Lamp, Agilent Technologies, Germany).

The data were recorded with ASTRA software (6.1.2, Wyatt Technology Europe, Germany). The data analysis was performed with the same software but with an updated version (6.1.7). BSA injections were performed to confirm good performance of the system. Blank injections were subtracted from the detector signal to prevent signal drift. The Berry method was used to extrapolate the data of the scattered angles located at 57.0–126 $^{\circ}\text{C}$. The chromatograms were divided into three fractions corresponding to different groups of substances (Krebs et al. 2017). Substances eluting in fraction 1 were proteins (22–32.7 kDa), in fraction 2 a mix of P-PC and LN-SP (42.7–65.9 kDa without one outlier, 121.4 kDa), and in fraction 3 HN-SP (2.63–25.07 $\cdot 10^3$ kDa). The dn/dc value used for molar mass determination was different for each fraction, using a value of 0.185 mL/g for fraction 1, while fractions 2 and 3 had a value of 0.146 mL/g. To avoid wording confusion, the term “concentration” is simply used to represent the calculated mass in each AF4 fraction calculated with DRI with μg as a unit.

The molar mass range in parenthesis for each fraction was established as a reference by only using the values of “relevant” beer samples (β -glucan content of malt inside the required specification for brewing proposes < 350 mg/L, ISO 65 $^{\circ}\text{C}$).

Sensory analysis

The beer samples were tasted by a trained sensory panel certified by the Deutsche Landwirtschafts-Gesellschaft, German Agriculture Society (DLG). The *PF intensity* and *quality* as well as *mouthfeel* (*watery*, *slimy*) descriptors were evaluated. In turn, the mouthfeel descriptors can function as PF quality indicators (Krebs, Gastl et al. 2020). As the name

suggests, *PF intensity* comprises how strong the sample is while *PF quality* how pleasant the sample is. All descriptors were rated on an intensity scale from 0 to 7. The beers were evaluated in nine different sensory sessions within a time span of one week. On average, nine panelists evaluated the beers in each session. Samples were tasted as fresh as possible, and they were tempered for one hour at room temperature before the tasting (15 $^{\circ}\text{C}$ drinking temperature). Tasting cups with a three-digit code were used to present the samples. Since the focus of this study is on the molar mass of starch and non-starch polysaccharides (macromolecular profile) and their effect on the perception of *PF* and *mouthfeel*, the panelists were instructed to wear nose clips to exclude the influence from volatile components.

Data analysis

Data analysis was performed on JMP Pro 15 software (version 15.2.0, SAS, Belgium). A non-parametric Wilcoxon test was used to identify differences between two sets of data. When more sets of data were present, e.g., barley modification characteristics, a non-parametric all pairs Steel–Dwass was used.

Human and animals rights

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

Results and discussion

Standard malt analysis

A total of 55 independent malt samples were produced. Two samples from variety E and G were not analyzed as not enough material was left after the brewing process; thus, SMA accumulated 53 samples in total. Different parameters describing amyolytic, proteolytic, and cytolytic aspects were analyzed (Fig. 1). Extract in malt presented variations from 79.1 to 88.1% w/v and final degrees of attenuation (FDA) from 82.1 to 93.1%. Meanwhile, SN and β -glucan ranges were 440–909 mg/100 g DM and < 20 –1504 mg/L, respectively (lowest detection limit for the latter). Because of the intention to focus on the effect of starch and non-starch polysaccharides on *PF* and *mouthfeel* in beer, the β -glucan content (regarding the cytolytic parameters) needs to be inside the recommended specification for brewing application to include them in the beer sensory evaluation (Sect. 3.4). β -glucan content in malt outside of the required specification (< 350 mg/L, ISO 65 $^{\circ}\text{C}$ mash) was found in 52.8% of the samples.

Fig. 1 Standard malt analysis of amyolytic (a), proteolytic (b), and cytolytic (c) parameters of barley malt samples ($n=53$); barley varieties classified as different modification characteristics (low, moderate, high) and modified by malting procedures (steeping degree as parameter) within the class at two levels (l; low, and h; high). The blue areas show barley malt quality guide values for ISO 65 °C (Back, et al., 2019). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

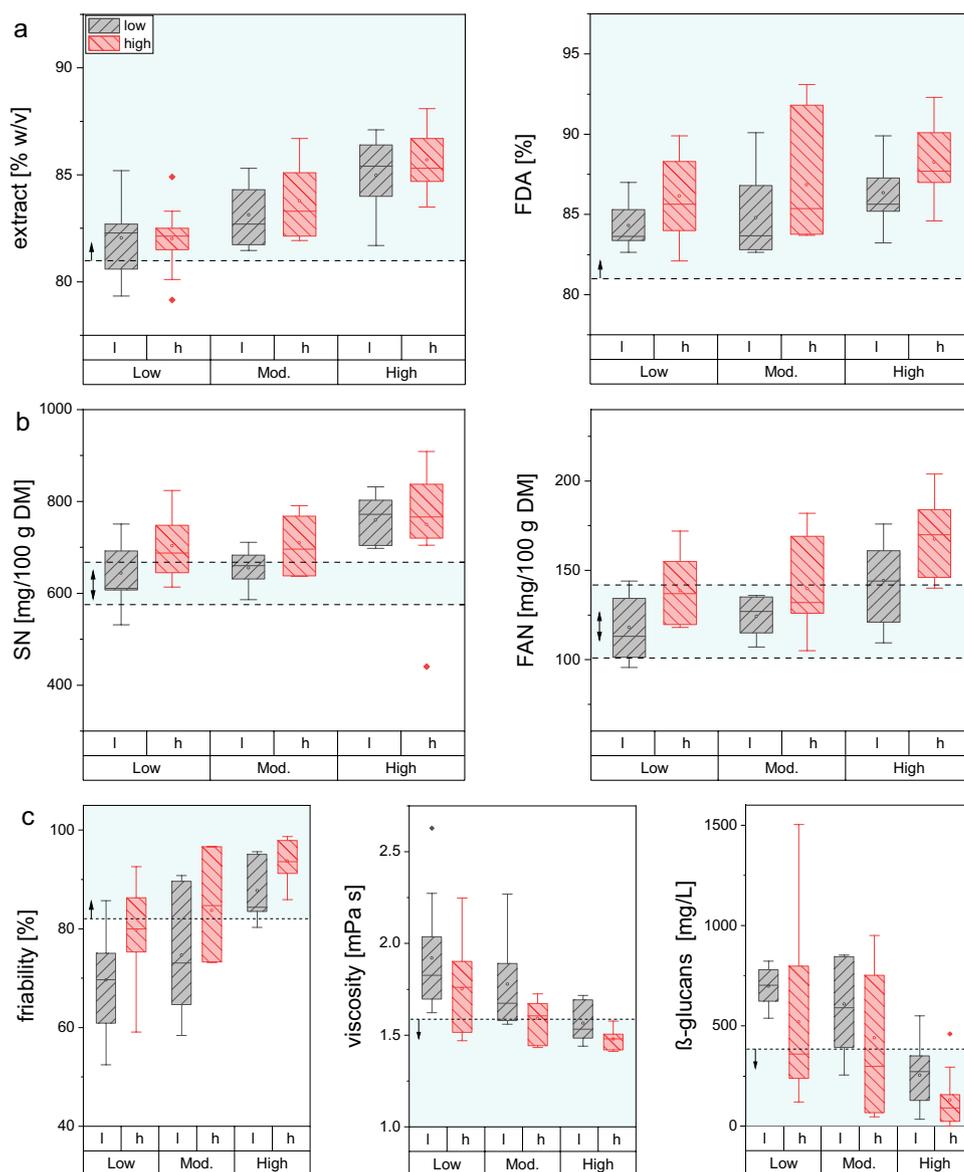


Figure 1 depicts the range of amyolytic (1a), proteolytic (1b), and cytolytic (1c) malt parameters (SMA) measured in barley malt produced from varieties classified as various modification characteristics classes (low, moderate, and high), each class at two different modification levels during malting. The effects of modification intensity (by steeping degree) on malt quality parameters will be discussed first. Within the same modification characteristic class (treatment effect), samples with the further modification level (high steeping degree) presented higher average values on FDA, SN, FAN, and friability. The opposite occurred for viscosity and β -glucan content where the higher values were present in malts produced with a low steeping degree. When comparing the effect of malt modification intensity among the various modification characteristic classes (sample effect

upon treatment), the average values of FDA, SN, FAN, and friability increased from low to moderate and high modification classes at both modification levels studied. Regarding viscosity and β -glucan, high modification class showed the lowest response followed by moderate and low modification classes on the modification intensities used.

Regarding responses due to barley malt modification characteristics (average response per class), amyolytic and proteolytic parameters increased their values according to their modification classification from low to moderate and high variety modification characteristics. The extract was significantly greater for the high modification characteristic class (85.3% w/v) compared to the moderate (83.5% w/v) and low (82.0% w/v) sample classes (p-value < 0.05, non-parametric test). Although there were no statistical

differences in average responses per modification characteristic class, the average FDA tended to be larger in high modification characteristic varieties following a similar trend as observed in extract parameters. FDA may be interpreted as an indicator for the fermentable part of low molecular weight sugars of (laboratory) wort (higher the FDA is, the fewer dextrans are present in the final product). Consequently, it may serve as an indirect practicable measure for (non-fermentable) dextrans. Thus, the results indicate that high modification characteristic varieties present lower dextrin content after malting.

On proteolytic parameters, the higher SN was found in the high modification characteristic class with 754.3 mg/100 g DM, followed by moderate and low modification samples averaging 684.5 and 675 mg/100 g DM, respectively, which was the first and only one statistically different (p -value < 0.05 , non-parametric test). The same behavior was observed in FAN parameter. As expected based on maltsters' expertise, the high modification class presented the highest friability in cytolytic parameters, 90.7%, and the lowest viscosity, 1.52 mPa s, and a β -glucan content of 191.1 mg/L (p -value < 0.05 , non-parametric). The moderate modification class had medium average values (79.2%, 1.68 mPa s, and 523.6 mg/L), while the low modification samples had the highest (1.83 mPa s, 604.3 mg/L) and lowest for friability (74.9%).

Research regarding malt quality is mainly focused on processability of the malting barley variety. Depending on specific amylolytic, proteolytic, and cytolytic quality parameters, an adequate malting and brewing performance should have been guaranteed. Influence of the malt quality on the sensory characteristics of the resulting fresh beer has remained largely unconsidered until now or has been focused on aging stability [36]. The variation in malting quality parameters demonstrates that a wide spectrum of various chemical compositions could be obtained by varying the malting barley variety (natural-based composition of the substance groups by variety and location) and modification level during malting (parameter steeping degree). Other research has also shown chemical variations in malt from various grain sources by changing different modification parameters [6, 15, 21, 31, 45].

Molar mass of beer (AF4-MALS-DRI)

55 beers were produced with the previously described barley malt samples. The samples were analyzed by AF4-MALS-DRI, from which three fractions were obtained representing different compounds in beer according to a literature method [20]. Size (Rms radius) could only be calculated for fraction 3, suggesting that fractions 1 and 2 had a radius smaller than 10 nm which is the detection limit for MALS detection [38]. The average molar mass range of all the samples

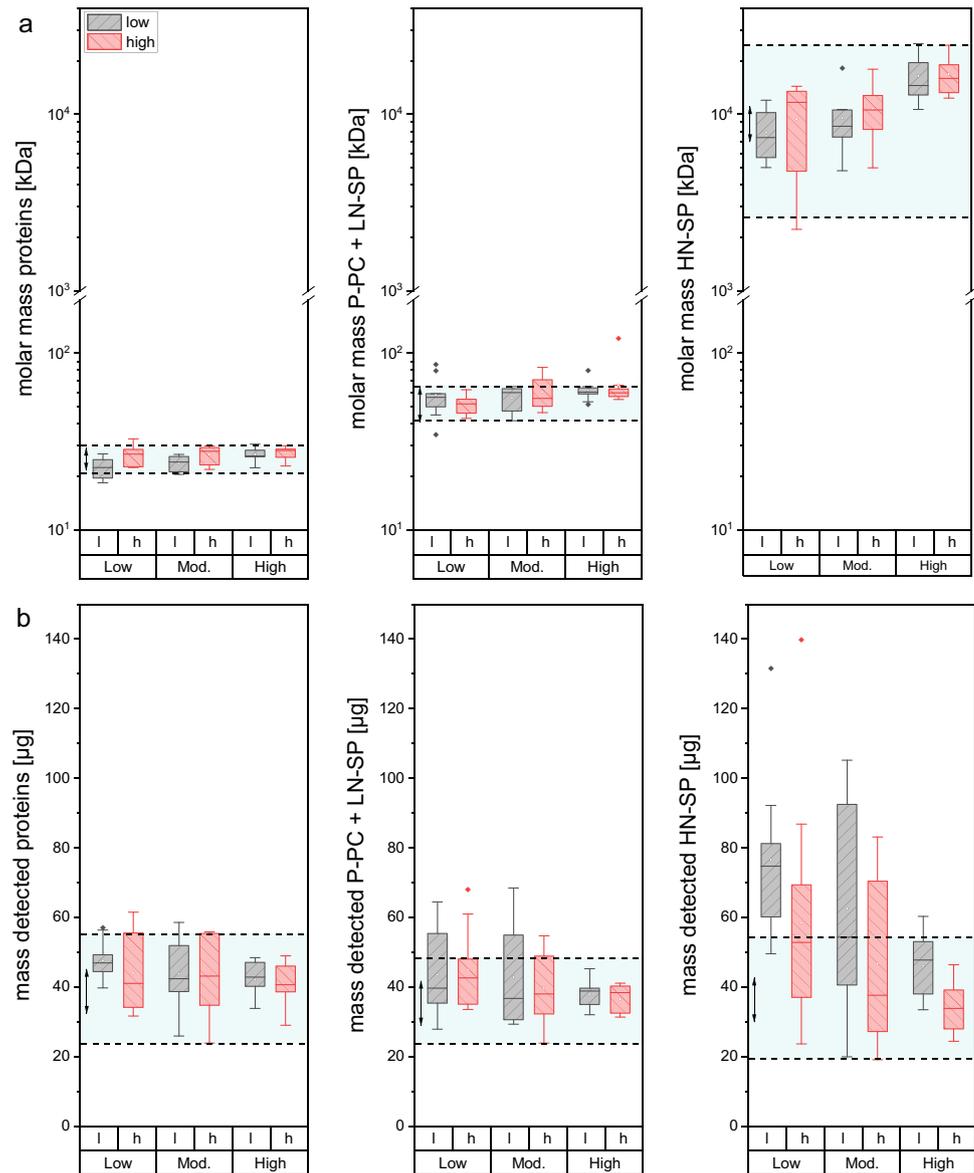
for protein fraction (1) varied from 18.45 to 32.7 kDa. The molar mass fluctuation of fraction 2 was 34.6 to 121.4 kDa. Molar mass of proteins in beer has been reported to be lower than 100 kDa, corresponding to fractions 1 and 2 of the AF4 [30]. The presentation of a molar mass range higher than 100 kDa in fraction 2 might be attributed to co-elution with non-starch polysaccharides containing a higher molar mass than the proteins, hence increasing the average molar mass to more than 100 kDa [48]. The broadest molar mass response was present in the HN-SP (3) with $2.23\text{--}25.08 \cdot 10^3$ kDa ($2.2\text{--}25 \cdot 10^6$ g/mol). Literature values for arabinoxylans ($2.3\text{--}12.6 \cdot 10^5$ g/mol [32]) and β -glucans ($< 2000\text{--}10^8$ g/mol [4, 49]) in malt/beer were inside the measured range.

Figure 2 shows the molar mass (2a) and mass detected (2b, AF4 fractions) of beers produced with previously shown barley malt. In general, the molar mass of proteins (fraction 1) and P-PC and LN-SP (fraction 2) presented a minor increase in samples brewed with high modification level compared to the low modification level in most of the AF4 fractions. This was not the case for the high modification characteristic class in which similar molar masses were observed in all fractions despite the difference in malting modification level.

When comparing the overall responses (average by modification characteristic class), no significant differences were observed for molar mass and mass detected. The molar mass of proteins (fraction 1) was the highest (27 kDa) for high modification varieties despite having the lowest mass detected in the AF4 (41.3 μ g). Since this class presented the highest SN according to SMA (further protein degradation, Fig. 1b), the formerly mentioned values in fraction 1 were not expected. During malting, the proteins are hydrolyzed. The common methods for protein quantification in the brewing industry are Dumas and Kjeldahl, which are techniques that determine to various extents the nitrogen content in the sample followed by the use of a conversion factor (6.25 for the brewing industry) [24]. Because the AF4 separation channel contains a 10 kDa membrane, most of the substances below this threshold will be lost during the fractionation. This suggests that the majority of SN measured by SMA might present a size smaller than 10 kDa and are lost during channel fractionation, also explaining the lower mass detected on malts with the highest protein degradation (high modification class).

The molar mass responses as well as the mass detected changed accordingly to the barley variety modification characteristics in all AF4 fractions, showing the high modification class as the highest (for molar mass) and lowest (for mass detected) values followed by the moderate and low modification variety classes. As an example, molar mass of HN-SP, in which amylolytic and cytolytic compounds are

Fig. 2 Molar mass (a) and mass detected (b) of the produced corresponding beers ($n=55$) detected by AF4 using barley malt samples (three classes of various modification characteristics and modified by malting procedure in two modification levels (steeping degree as parameter, see Table 1). Beers were fractionated into three fractions by AF4: F1, proteins (22–32.7 kDa); F2, P-PC and LN-SP (42.7–65.9 kDa); and F3, HN-SP (2.63–25.07·10³ kDa). Dextrins might be present in all fractions. Values in parentheses show the molar mass of brewing relevant samples also highlighted on blue in the graphs (β -glucan content in malt < 350 mg/L, ISO 65 °C). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)



present, was statistically (p -value < 0.05, non-parametric) the highest for high modification characteristics varieties with an average value of $16.67 \cdot 10^3$ kDa. Medium ($10.04 \cdot 10^3$ kDa) and low ($8.69 \cdot 10^3$ kDa) modification variety classes presented statistically similar values.

Among the analytical malt quality parameters, cytolytic criteria (β -glucans, viscosity, friability) are key parameters to prevent problems during beer production (e.g., beer filtration). During the malting and brewing processes, cell wall polysaccharides are degraded, decreasing their concentration and molar mass [14, 25]. This behavior was also found in this work but only with the concentration parameter since the mass detected by the AF4 of cytolytic fractions, fractions 2 and 3, were decreasing according to their modification characteristics classes and levels of modification (by steeping degree). The samples (high modification characteristic

class) expected to contain the lowest molar mass on the aforementioned fractions, as the malts showed more degradation according to SMA (Fig. 1c), presented the highest molar mass (fraction 3, $2.63\text{--}25.07 \cdot 10^3$ kDa). There might be two possible explanations for these results. An interaction between β -glucan and arabinoxylans in fraction 3 might happen, increasing its molar mass [17]. The second explanation might be related to the co-elution of dextrins on all fractions that might yet intervene with the molar mass determination limiting the molar mass of beer by AF4 without any pre-treatment. If cytolytic substances are degraded more extensively than dextrins during the brewing process, the higher molar mass of dextrins will hinder the low molar mass of β -glucans in the AF4 fractions increasing the molar mass response as observed with other high molar mass polysaccharides [37]. This effect might also explain the higher

molar mass observed for protein fraction in high modification characteristic class as previously discussed. Although high molar mass polysaccharides have been found in beer with AF4 [47], the majority of beers' dextrans currently reported in literature are in the range of 5–27 DP [7]. Still, enzymatic degradation of dextrans in AF4 chromatograms confirms their presence in all fractions [20].

Dextrans are dispersed in all AF4 fractions. Thus, the accumulative mass detected of all the fractions by AF4 might be a relevant indicator for dextrin concentration. Despite the fact that other substances are also present in accumulative mass detected and influence this response (e.g., β -glucans or proteins), carbohydrates are the major beer component from which could take up to 50 g/L showing that dextrans are present in greater amounts than N-SP in beer produced from barley malt [27, 28]. Furthermore, a negative correlation between the accumulative mass detected and FDA was observed in the samples (-0.712 , p -value < 0.001 , $n = 53$), confirming the relation between FDA (dextrin content) and the total of all concentrations from AF4. It is important to remark that this assumption is done with data from standardized wort analysis and not the beers. As the mashing temperatures from SMA and the beers are different, the FDA would not be the same but it can still provide a similar result to what is expected [35].

The accumulative mass detected by AF4 per the variety modification characteristic class is depicted on Fig. 3. The low modification characteristic class averaged the highest accumulative mass detected by AF4 (157 μg) followed by the moderate (139.3 μg) and high modification characteristic

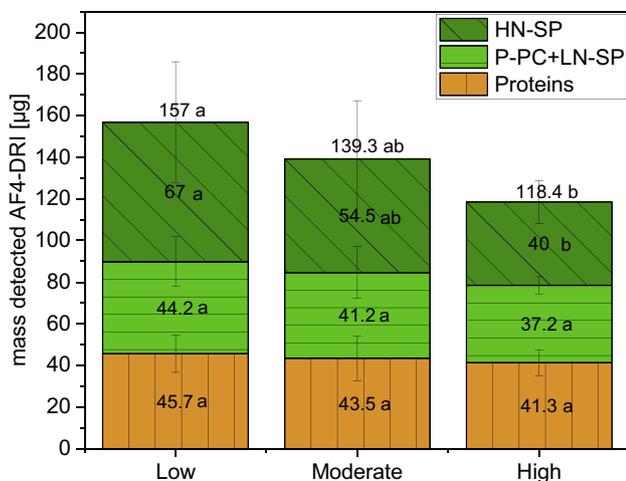


Fig. 3 Mass detected of different AF4 fractions and their accumulative values by differential refractive index (DRI) detector of all beer samples ($n=55$). Different letters represent statistical differences separated by barley modification classes (low, moderate, high) by Steel–Dwass all pairs non-parametric test. The error bars represent the standard deviation. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

varieties (118.4 μg). From SMA (Fig. 1a), it was suggested that the high modification characteristics class presented the highest FDA, suggesting a low dextrin concentration. Thus, the combination of a low accumulative mass detected by AF4 and high FDA by SMA sustains the claim that the samples of the high modification characteristics class contains fewer dextrans compared to the low and moderate modification characteristic classes. A comparable behavior was present when each concentration fraction was analyzed independently, but only fraction 3 showed significant differences. This suggests that HN-SP (fraction 3) is more susceptible to change due to barley (variety) characteristics and levels of malt modification (steeping degree). Yet, it is important to recall that substances lower than 10 kDa are not measured due to the ultrafiltration membrane in the separation channel (similar to the protein fraction previously explained).

Molar mass differences in beer produced by barley malt with focus on β -glucan brewing specification

With the chosen experimental approach, the goal was to obtain a broad range of β -glucan responses in beer based on the malt sample substance groups. However, just 47.2% of the malt samples fulfill the required brewing specification (β -glucans in malt < 350 mg/L, ISO 65 °C) and are thus suitable for brewing purposes. Regarding sensory perception, retrials confirmed this substance group increases the sensory perception of sliminess in beer [23].

When comparing the relevant cytolytic AF4 fractions, HN-SP (fraction 3) presented a higher molar mass in samples within the β -glucan specification compared to samples outside of this requirement (Fig. 4a). Moreover, samples within β -glucan specification presented a significantly higher FDA (88.1% compared to 84.3% of samples outside of the specification, p -value < 0.05) suggesting fewer dextrans. Consequently, the accumulative average mass detected of AF4 fractions was lower (114.4 μg) for samples within the β -glucan specification and reaching up to 163.1 μg in samples outside of this specification (p -value < 0.05). Furthermore, the AF4 fractions presented different mass detected as shown in Fig. 4b. It is noticeable that the concentration difference between samples within and outside of the β -glucan specification ($\Delta F_x = \text{mass } F_x \text{ outside} - \text{mass } F_x \text{ inside}$, x representing the same AF4 fraction) is different in the AF4 fractions. HN-SP showed the highest difference with $\Delta F_3 = 32$ μg compared to the proteins and P-PC and LN-SP with $\Delta F_1 = 6.4$ and $\Delta F_2 = 10.4$, respectively. These Δ values indicate degradation in AF4 fractions due to malt modification levels, which is perceptible in the final beer (> 10 kDa). Interestingly, HN-SP (fraction 3, $2.63\text{--}25.07 \cdot 10^3$ kDa) had the biggest Δ value suggesting it to be more susceptible to degradation during malting. Research from Chen et al. demonstrates also that high molar mass dextrans are

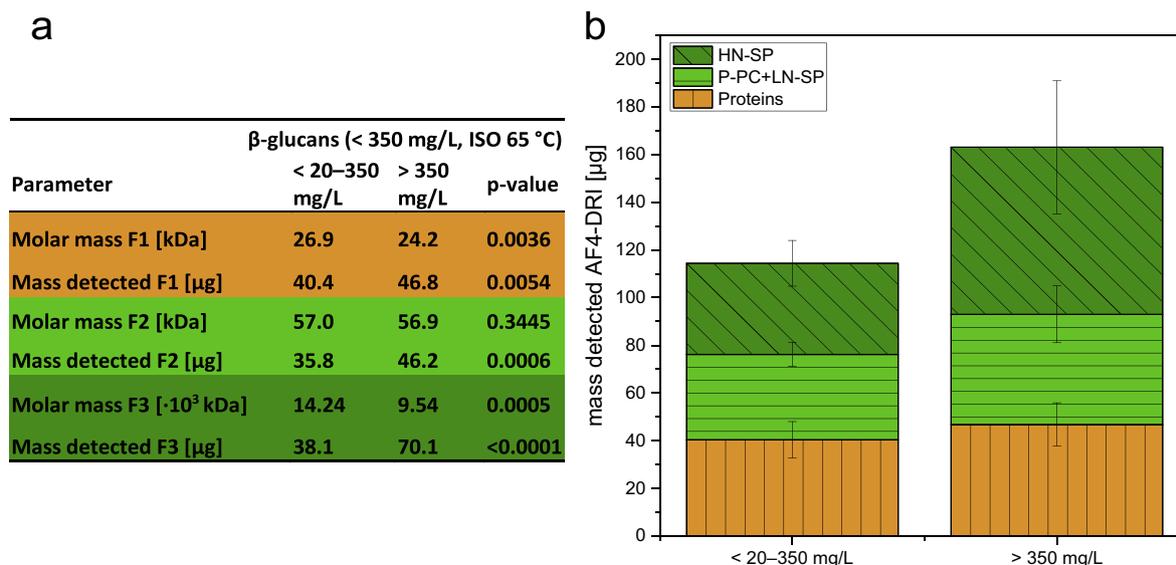


Fig. 4 Comparison of molar mass and mass detected by AF4 of beer samples within ($n=25$) and outside ($n=28$) β -glucan specification (<350 mg/L, ISO 65 °C). **a** Mean comparison by Wilcoxon non-parametric test. **b** Graphical comparison of average concentra-

tion of three fractions detected by AF4-DRI. The error bars represent the standard deviation. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

enzymatically digested primarily than small dextrans [3]. Since carbohydrates are the major non-volatile component, it may be suggested that a similar effect occurs during barley malt modification, thus explaining the high Δ values, hence higher degradation, in the high molar mass fraction in malt and, consequently, beer.

Working with samples outside of brewing specification, despite being of scientific interest, is not part of the scope of this paper. Therefore, only samples within the brewing specification are relevant for further analysis to evaluate physico-chemical responses of beer based on malt samples and its relation to sensory perception of *PF* and *mouthfeel*. However, it is not possible to compare sensory results with regard to the influence of modification class (variety characteristic) since the data set is not uniform. 60% ($n=15$) of the samples within β -glucan specification (<350 mg/L in malt, ISO 65 °C) were composed of high modification characteristic varieties while both moderate ($n=5$) and low ($n=5$) modification characteristic varieties of 20% each made statistical analysis unequal and too small to be compared.

Sensory responses of beer based on malt samples within specification (β -glucans < 350 mg/L, ISO 65 °C)

From this point on, the results are solely composed of barley malt samples within specification for malting barley to guarantee good brewing processability (β -glucans <350 mg/L in malt). The aim of this study is to explain which fractions (mass detected and molar mass)

and substances could positively (or negatively) affect the sensory impression (*PF* and *mouthfeel*) of beer. In terms of the formulated hypothesis, special attention was focused on β -glucans and dextrans because of their known negative and positive sensory perceptions, respectively. Regarding samples within specification, the samples covered all of the spectrum of β -glucan content. The sample distribution with a β -glucan content between $n < 20$ and 100 mg/L was 36%, between 100 and 220 mg/L 28%, and between 220 and 350 mg/L 36%. This allowed the study of the relation among SMA, molar mass, and sensory responses of beer samples at all possible cytolytic (β -glucans) modification levels within specification.

A sensory analysis of the produced beer samples was performed. As expected, *PF intensity* presented a negative correlation to a *watery* descriptor (-0.482 , p -value <0.05). Diversely, *PF quality* was negatively correlated to both *watery* (-0.407 , p -value <0.05) and *slimy* (-0.314 , p -value 0.126). These correlations between *PF* and *mouthfeel* descriptors exhibit their synergetic relevance. *Watery* has previously been reported as a negative *mouthfeel* descriptor to *PF intensity* in bottom fermented beer. Still, no positive correlation was observed between *mouthfeel* descriptor *slimy* and *PF intensity/quality* [22]. In samples with <350 mg/L of β -glucan content (recommended brewing specification) *PF quality* was influenced by *slimy* and *watery* mouthfeel descriptors. Therefore, this suggests that *PF quality* can indeed be analyzed as an independent descriptor. This confirms that it is possible to generate a high *PF intensity* beer with low (unpleasant) *PF quality* (*slimy*). How this

influences consumers' preference could be the subject of insightful future research.

A minor positive correlation was found within *PF* descriptors (*intensity-quality* 0.351, *p*-value 0.086) but not within *mouthfeel* descriptors (slimy–watery 0.174, *p*-value 0.405). The relation between the *mouthfeel* descriptor *slimy*, β -glucan content, and FDA (dextrins) is depicted in Fig. 5. Beer produced from highly attenuated malt presented a higher *sliminess* despite presenting a low β -glucan content. This effect could be attributed to the low dextrin content in these samples (Fig. 3). Due to its absence, the sensory perception caused by β -glucans is dominant (synergistic or masking effects). Contrary to this, literature suggests that the presence of β -glucans positively influences sliminess [23]. However, there were some important differences. The matrix used was different (non-alcoholic beer). Undoubtedly, ethanol influences the matrix perception [16, 41]; in addition, Krebs et al. worked with the same non-volatile matrix (spiked sample), while this study had samples with different physico-chemical characteristics, assessed by SMA.

In conclusion, it can be suggested that in beer samples with a different non-volatile matrix the balance of β -glucans and dextrins is a key factor for the *mouthfeel* response (*sliminess*) and consequently the (*PF*) *quality* of the product. In turn, this effect is dependent on barley modification characteristics (modification class) as well as with a modification level caused by a malting procedure.

To point out the relation between β -glucans/FDA to molar mass response and *PF*, the samples within β -glucan specification were classified according to their concentration as low (< 20–100 mg/L, *n* = 9), moderate (100–220 mg/L,

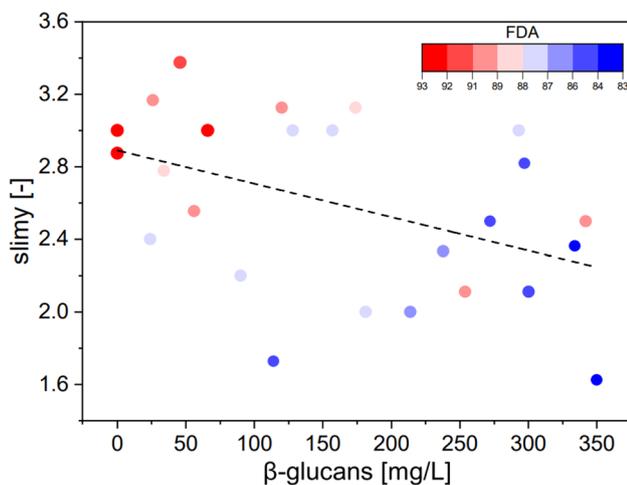


Fig. 5 Relation between the descriptor *slimy*, β -glucans, and final degree of attenuation (assumed as reference to dextrins) of beer samples within cytolytic malting barley specification (β -glucans in malt < 350 mg/L, ISO 65 °C, *n* = 25). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

n = 7), and high (220–350 mg/L, *n* = 9) β -glucan content. Subsequently, the FDA of the samples decreases as β -glucan concentration increases: $90.4 \pm 2.1\%$ for low β -glucan, $87.4 \pm 1.5\%$ moderate β -glucan, and $86.6 \pm 2.3\%$ high β -glucan. Although *PF intensity* had a minor tendency to decrease upon an increase in β -glucan concentration, the overall responses of this descriptor were similar (Fig. 6a). However, *PF quality* was the lowest in samples with β -glucan content < 100 mg/L and a high FDA (less dextrins content), increasing this descriptor values for moderate and high β -glucan content. This suggests that a moderate β -glucan content, 100–350 mg/L, and FDA, < $87.4 \pm 1.5\%$, improve the quality of beer. No clear relationship between molar mass and sensory responses of samples classified by β -glucan content was observed (Fig. 6b). High β -glucan content samples averaged the lowest molar mass of HN-SP ($12.41 \cdot 10^3$ kDa), while moderate ($15.45 \cdot 10^3$ kDa) and low ($15.03 \cdot 10^3$ kDa) samples presented similar values.

Molar mass relevance of beer with similar chemical characteristics

Whereas barley varieties of high modification class are characterized as low β -glucan content and intensive modification behavior, β -glucan content can be enriched by a moderate malting regime (low malt modification). Conversely, this also applies to low modification varieties. In order to analyze the influence of variety modification characteristic, data of beer samples produced from barley malt with similar β -glucan and dextrin content was used and their resulting molar masses were compared. Four samples presented similar responses (β -glucan and FDA, related to dextrins) at different concentrations. Two samples, low (G) and high (A) modification characteristics classes, showed an average β -glucan and FDA content of 165.5 mg/L (± 12) and 88.2% (± 0.1) in malt, respectively. Accordingly, the other two samples, moderate (E) and high (C) modification characteristics, registered values of 298.8 mg/L (± 2.1) and 85.5% (± 0.2). The high modification characteristic varieties were grouped, and their molar mass and sensory characteristics were compared to the low and moderate modification characteristic varieties as shown in Table 2. The sensory characteristics and molar mass of these samples are depicted in Table 2. In samples with similar dextrin and β -glucan content, the molar masses were different in all AF4 fractions. High modification characteristic varieties had the highest molar mass in HN-SP (fraction 3, $2.63\text{--}25.07 \cdot 10^3$ kDa), while proteins (fraction 1, 22–32.7 kDa) and P-PC and LN-SP (fraction 2, 42.7–65.9 kDa) fractions were higher with low-moderate modification characteristics (L-M). Hence, these results demonstrate that you may have similar concentrations in the liquid matrix, but the molar mass could be different due to different characteristics of the raw materials used.

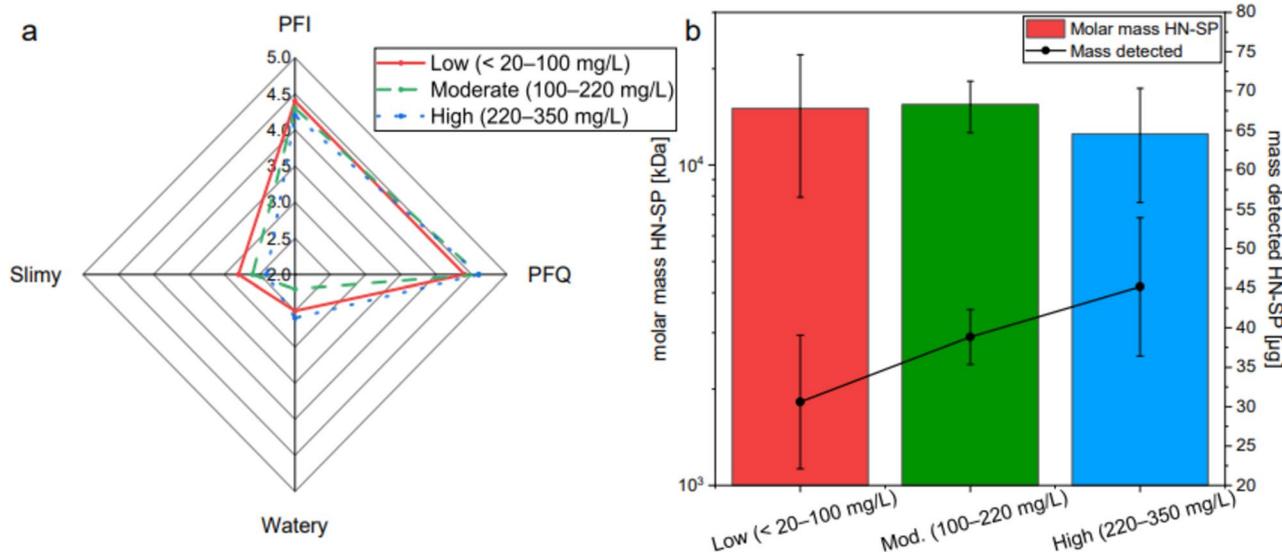


Fig. 6 Sensory results (a) and molar mass of HN-SP (fraction 3 AF4, $2.63\text{--}25.07\cdot 10^3$ kDa) (b) of beer classified by β -glucan concentration (in malt, ISO 65 °C) in low ($90.4\pm 2.1\%$ FDA, $n=9$), moderate ($87.4\pm 1.5\%$ FDA, $n=7$), and high ($86.6\pm 2.3\%$ FDA, $n=9$); only beer produced from malt samples with a β -glucan concentration

$n < 350$ mg/L was considered ($n=25$). The error bars represent the standard deviation. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article)

Regarding sensory evaluation, high modification characteristic varieties present a higher *PF intensity* while L-M samples are more *slimy*. Despite presenting a similar accumulative concentration from AF4 fractions, the fractions presented different distributions. L-M samples had a higher concentration of protein (fraction 1, $22\text{--}32.7$ kDa) but lower in HN-SP (fraction 3, $2.63\text{--}25.07\cdot 10^3$ kDa). Higher concentration of HN-SP in high modification characteristic samples combined with a higher molar mass might explain the *PF intensity* increase. This is in line to RübSam et al. (2013), in which concentration and molar distribution of dextrans positively influence the *PF intensity* of beer. Despite having the presence of different substances in HN-SP, it can be suggested that dextrans and β -glucans with a molar mass around $2.63\text{--}25.07\cdot 10^3$ kDa influence the *PF* and mouthfeel of beer. Therefore, molar mass is a relevant indicator in addition to concentration for assessing the quality of beer.

To sum up, it was shown that molar mass is a key factor for the assessment of the *PF* and *mouthfeel* responses on beer. This work demonstrated that AF4 can be a valuable technique to study physico-chemical characteristics of beer based on different raw material matrix compositions. Molar mass and mass detected by AF4 in beer varied among the modification class of a variety used, with a variety of low modification characteristic class the highest mass detected, followed by moderate and high modification characteristics. The broadest molar mass variation was observed in HN-SP (fraction 3, $2.63\text{--}25.07\cdot 10^3$ kDa), which in turn, was more

Table 2 Comparison of four standardized brewed beer samples with malted barley with similar β -glucan and final degree of attenuation (FDA), but assigned to different modification characteristic classes (low to moderate against high variety-dependent modification characteristic)

	Unit	L–M ($n=2$)	High ($n=2$)
PFI	(-)	4.3 ± 0.3	4.7 ± 0.5
PFQ	(-)	4.5 ± 0.3	4.5 ± 0.2
Watery	(-)	2.6 ± 0.5	2.4 ± 0.8
Slimy	(-)	3 ± 0.2	2.6 ± 0.6
Molar mass F1	(kDa)	30.7 ± 2.8	26.9 ± 1.6
Mass detected F1	(µg)	48 ± 9.9	40.2 ± 2.1
Molar mass F2	(kDa)	62.6 ± 0.6	58 ± 1.6
Mass detected F2	(µg)	37.1 ± 0.5	37.8 ± 4
Molar mass F3	($\cdot 10^3$ kDa)	12.30 ± 2.34	16.69 ± 4.80
Mass detected 3	(µg)	38.8 ± 0.9	47.1 ± 1
Accum. mass detected	(µg)	123.8 ± 11.2	125.1 ± 0.8

susceptible to molar mass changes due to malting (parameter steeping degree).

When samples with less than 350 mg/L of β -glucan content in malt are considered, the balance of β -glucans and dextrans, indirectly measured by FDA in SMA, was important. A FDA higher than 87.4% decreased the *PF quality* of beer since its *sliminess* (negative) increased despite presenting a β -glucan content lower than 100 mg/L. In beer samples brewed with malt samples with the same β -glucan and FDA

content, higher molar masses of HN-SP of high modification characteristic varieties explain the improvements (*PF intensity* increase) in the sensory characteristics of beer. This confirms the importance of FDA as a practical parameter easily assessed in praxis.

However, the lack of dextrans was appointed as the main factor to explain the difference in *sliminess* of low β -glucan content samples. Research has suggested dextrans as a positive *PF* precursor by increasing the *intensity* and *quality* of cereal-based beverages depending on concentration and molar mass [23, 43]. In consequence, it is assumed that at a low dextrin concentration, β -glucans could be sensory dominant; hence, a *slimy* perception is present in these samples.

Conclusions

The molar mass response of barley malt samples could be influenced by their modification characteristics of the variety and the modification intensity caused by malting procedure (parameter steeping degree). Whereas barley varieties from a high modification characteristic class are characterized by low β -glucan content and intensive modification behavior, the β -glucan content can be enriched by a moderate malting regime (low modification level). Conversely, this also applies to low modification varieties. Working with different barley malt matrix compositions allowed us to produce beer with different physico-chemical characteristics. Varieties from a high modification characteristic class presented the lowest concentration in the three different AF4 fractions, but their molar mass was higher. This was attributed to a molar mass determination limitation since AF4 fractionates by size and same size substance; however, different molar masses (e.g., arabinoxylans and dextrans) might be co-eluting. HN-SP (2.63–25.07·10³ kDa), where cytolytic and dextrin substances are enclosed, presented the most concentration change (Δ) on malt modification intensity compared to protein (22–32.7 kDa) and P-PC and LN-SP (42.7–65.9 kDa) fractions. Varieties within a high modification class tend to contain very low β -glucan content (below detection limit < 20 mg/l according to MEBAK); however, this result suggests that moderate β -glucan content (100–350 mg/L in malt) in combination with dextrans (< 87.4 ± 1.5% FDA) improves the *PF quality* perception of bottom fermented beers. Therefore, excessive modification of malt could negatively influence the final product.

β -Glucans and dextrans are interconnected to high molar mass compounds that can be altered during the malting process. Although no clear molar mass-sensory relation was observed due to co-elution, it was demonstrated that a difference in molar mass was relevant for changing the sensory perception of bottom fermented beers at the same concentration of dextrans and β -glucans. Consequently, this sensory

change was possible due to the different modification characteristics of the raw materials used. Future research should focus on elucidating the single role of substances in HN-SP (β -glucans, arabinoxylans, and dextrans). This will confirm the sensory impression of starch and non-starch polysaccharides to fully understand the mechanisms behind sensory quality, which in turn may aid brewers to produce better cereal-based beverages.

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Declarations

Conflict of interest The authors declare no conflict of interest.

Compliance with ethics requirements All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

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