Influence of malt modification and the corresponding macromolecular profile on palate fullness in cereal-based beverages

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Abstract
The sensory attribute palate fullness of cereal-based beverages was shown to be affected by polymeric compounds and their macromolecular profile. During malting, the enzymatic degradation of polymers is technologically controlled by the malting parameters, namely the degree of steeping, germination time, and germination temperature. The macromolecular profile of a fermented cereal-based beverage consists of non-fermentable substance classes. Therefore, the macromolecular composition of a final beverage is originally dominated by the raw material, if conventional production methods are used. We investigated the influence of different cytolytic and proteolytic malt modifications on the macromolecular profile of lactic acid-fermented cereal-based beverages (a strain was selected that did not produce exopolysaccharides) and their resultant effect on the sensory perception of the attributes of palate fullness and mouthfeel. Asymmetrical-flow field-flow fractionation coupled with multi-angle light-scattering detection and refractive index detection is an analytical tool for macromolecular characterization to indicate differences in the macromolecular profile, molar mass, and molar mass distribution. The beverages produced using different modified malts demonstrated a considerable variation in their final composition, particularly in the composition of their macromolecular compounds. A higher level of malt modification led to a decrease in the high-molar-mass fraction and a consequent shift toward fractions with a lower molar mass. Malts produced from barley with increased crude protein contents resulted in a greater range within the macromolecular profile. The variation of germination time significantly influenced the number average molar mass, the total refractive index detection (dRI) peak area, and the high-molar-mass fraction, which contained cell wall polysaccharides (60–1200 kDa). The perception of the intensity of palate fullness was significantly correlated with specific macromolecular fractions, which were influenced by the malting parameter degree of steeping and the resultant modification. The perception of the mouthfeel descriptor watery varied significantly for different crude protein contents. Our results are beneficial for a targeted design of beverage composition based on the macromolecular profile by an improved selection of raw materials and malting technology.

Keywords AF4 · FFF · Palate fullness · Malt modification · Cereal-based beverage · Sensory description

Introduction
Palate fullness and mouthfeel are sensory quality parameters and key attributes for consumer acceptance and the drinkability of cereal-based beverages. Many types of cereal-based beverages are commonly available, and they differ in the type of fermentation used in the production process, being either alcoholic yeast fermentation, lactic acid fermentation, or mixed fermentation of both types. Palate fullness and mouthfeel have first been studied by Lewis and coworkers in the 1990s [4–6]. Generally, the perception of the intensity of palate fullness and mouthfeel is intensely affected by the macromolecular profile of the cereal-based beverage [1–6]. As well as total macromolecular concentration,
different non-volatile substance classes and their molar mass fractions also affect the perception of palate fullness and mouthfeel [3–17].

The macromolecular profile of cereal-based beverages is generally determined by polymeric compounds (proteins, polysaccharides, and polyphenols) and their progress in depolymerization during processing [1, 2, 18–20]. Since yeasts or specific strains of lactic acid bacteria (LAB) cannot metabolize high-molar-mass substances [21], the macromolecules are solely depolymerized during the mashing and mashing process by the malt’s intrinsic enzymes. Apart from the precipitation of polymers during boiling (trub formation), the macromolecular profile remains relatively unmodified during fermentation. The depolymerization processes are classified into cytolysis (degradation of cell wall polysaccharides), proteolysis (degradation of proteins into amino acids and peptides), and amylolysis (degradation of starch into fermentable carbohydrates) [13]. Dextrins are derived from the partial hydrolysis of starch (α-1-4- and α-1-6-linked d-glucose monomers) and vary in their molar mass [3, 22]. Hemicelluloses are cell wall polysaccharides derived from the barley cell wall during cytolysis that consist of 80–90% β-glucans (β-1-3- and β-1-4-linked glucose monomers) and 10–12% pentosans [23, 24]. Different proteins have been identified in cereal-based beverages, such as protein Z (40 kDa), LTP1 (9.7 kDa) and hordein-derived polypeptides (10–30 kDa) [16]. They are often chemically modified by hydrolysis or glycosylation during the Maillard reaction [16]. An estimation of the degree of proteolysis and the depolymerization products of proteins in malt can be characterized analytically by their soluble nitrogen and free amino nitrogen (FAN) content. In addition, the Kolbè index is calculated by the ratio of soluble nitrogen in relation to total protein content [16].

During malting, the enzymatic degradation of polymers is technologically controlled by the degree of steeping, germination time, and germination temperature [25–27]. Modern brewing barley varieties are bred to be balanced in malting performance and to meet the required brewing specifications. It assures optimal processability, especially with regard to practice-relevant high-short mashing regimes (amylolysis) [28]. Therefore, besides enzyme synthesis and release, the main objective of malting is the degradation of macromolecular compounds via cytolytic and proteolytic modification. In contrast, the degradation of starch into fermentable sugars (amylolysis) is the primary objective of mashing (substrate production for fermentation) for modern barley varieties and highly modified malts [25]. During the subsequent fermentation process, only low-molar-mass compounds [fermentable sugars and low-molar-mass proteinaceous compounds (FAN)] are metabolized by microorganisms [13, 25, 35] (e.g., LAB and yeasts).

Asymmetrical-flow field-flow fractionation (AF4) coupled with multi-angle light-scattering detection (MALS) and refractive-index detection (dRI) has been shown to be a suitable tool to analyze the macromolecular profile of complex matrices in cereal-based beverages [1, 2, 18–20]. Different substance classes have been identified within the fractogram fractions of cereal-based beverages, namely, proteins (fraction 1), protein-polyphenols (fraction 2), cell wall polysaccharides (fraction 3), and dextrins, eluting throughout all fractions [18]. Using AF4-MALS, a high range of variation within the macromolecular profiles was shown for commercial German lager beers and for non-alcoholic beers, which was attributed to different production methods [1]. Although the macromolecular profiles of the fermentation substrate and of the final beverage were shown to be technologically influenced by different raw materials and mashing profiles [19, 29, 30], the effect of different malt modifications has not been systematically studied.

Thus, the objective of this study was to characterize the effect of different cytolytic and proteolytic malt modifications on the macromolecular profile of cereal-based beverages produced by different malting procedures and their effect on the sensory perception of palate fullness and mouthfeel, without a change in the substrate production (mashing) process.

A further novel aspect of this research was the focus on lactic acid-fermented cereal-based beverages. Previous publications have studied the effect of macromolecular profiles in the matrix of lager beers [1] and non-alcoholic beers [2]. However, in the case of lager beers, the formation of ethanol by yeast fermentation also influences the perception of palate fullness and mouthfeel [1, 5]. In this study, we used lactic acid bacteria (Lactobacillus amylolyticus TL 5) to ferment the substrate without the formation of ethanol [21]. L. amylolyticus TL 5 was specifically chosen because it did not produce exopolysaccharides during fermentation [31].

Consequently, this approach is independent of individual beverage matrices and focuses on the non-fermentable and non-volatile compounds in the macromolecular fractions of the final beverage. The high range of variation of the raw material (malt modification) was adjusted to produce beverages with a wide range of macromolecular concentrations and molar masses, which assume a high range of variation in the intensity of palate fullness and in the quality of mouthfeel. Further, this proceeding is also applicable for other cereal-based beverages (e.g., beer), since the beverage production procedure and fermentation remain constant (as is common practice) and do not affect the macromolecular profile of the final beverage. The adaptation of malting parameters is useful for the technological control of macromolecular substance classes to influence the sensory perception of the intensity of palate fullness and mouthfeel.
Materials and methods

Malting procedure

Two malting barley samples of the variety Marthe (Saaten Union, Germany) were chosen that differed in their crude protein content (9.9% and 11.7%, respectively). Both barley were malted in a micromalting plant in small batches (sample amount: 1 kg) following MEBAK procedures for micromalting (R-110.00.008) [32], with variations in the degree of steeping (42% or 48%), germination temperature (12 °C or 18 °C), and germination time (5 days or 7 days) to cover a great spectrum of different modification levels. All samples were kilned standardized for 50 °C/16 h, 60 °C/1 h, 70 °C/1 h and 80 °C/5 h. Rootlets and sprouts were removed before further analysis. Malting experiments were done in duplicate.

Malt analysis

Amylolytic (extract R-205.01.080), proteolytic (soluble nitrogen R-205.11.030 and FAN R-205.14.111), and cytolytic (friability R-200.14.011, viscosity R-205.10.282, and β-glucan-content R-200.26.174) malt quality parameters were analyzed according to MEBAK procedures [32]. For the production of laboratory mash, the malt samples were dry milled in a laboratory mill with a gap of 0.2 mm (LM 3100, Perten Instruments, Sweden), and the ground malts were isothermally mashed at 65 °C for 1 h according to the MEBAK procedure R-207.00.002 [32]. Extract, soluble nitrogen, FAN, viscosity, and β-glucan-content were measured from laboratory wort according to MEBAK procedures [32].

Production of cereal-based beverages

The malts were milled in a laboratory mill (LM 3100, Perten Instruments), and the sample material was isothermally mashed at 65 °C for 1 h according to MEBAK procedure R-207.00.002 [32], with minor modifications. Briefly, 50 g of ground malt was mixed with 350 ml of distilled water (65 °C) and mashed at 65 °C. Then, 50 ml of distilled water (65 °C) was added after 30 min, and mashing was continued isothermally for a further 30 min (65 °C). The samples were subsequently cooled to room temperature, and the total mass was adjusted to 450 g with the addition of distilled water. The samples were centrifuged (15 min, 4400 rpm), and the supernatants were decanted from the spent grain. The standardized worts that were produced (substrate) were heated to 85 °C for 1 min for enzyme inactivation and subsequently cooled to 48 °C for further fermentation.

Lactobacillus amylolyticus strain TL5 was chosen for fermentation because it does not produce exopolysaccharides [31]. The LAB were propagated in 10% non-hopped malt extract (Weyermann®, Germany) and then washed with sterile quarter-strength Ringer’s solution (4400 rpm, 10 min) before fermentation. The inoculation rate was adjusted to 10 × 10⁶ cells/ml, and wort fermentation (2 l) was performed under static conditions (48 °C, ca. 21 h, up to equivalent pH levels within each group to be comparable). Then, the fermented beverages were immediately cooled to 5 °C, and the LAB were removed by centrifugation (15 min, 4400 rpm). The cereal-based beverages were produced in duplicate and were frozen until further analysis.

Composition analysis of cereal-based beverages

The chemical composition of the cereal-based beverages was analyzed using standard procedures according to MEBAK guidelines [32] for original gravity, dynamic viscosity (measured using a Stabinger rotational viscometer, from Anton Paar, Austria), total nitrogen, β-glucan (see malt analysis procedures), and pH (R-205.06.040) [32].

Macromolecular characterization of cereal-based beverages

The macromolecular profiles of the cereal-based beverages were characterized using an Eclipse AF4 (Wyatt Technology Europe, Germany) that separates macromolecules by their diffusion coefficient based on asymmetric-flow field-flow-fractionation. The AF4 was coupled online to a MALS DAWN HELEOS detector (Wyatt Technology Europe) and to a refractive index detection using an Agilent 1260 Infinity Refractive Index Detector (Agilent Technologies, Germany). The setup included an Agilent 1100 series isocratic pump (Agilent Technologies) for eluent delivery (composition: 50 mM NaNO₃ + 0.025% NaN₃; filtration by an internally placed Supor 0.1-µm membrane filter [Pall Corporation, USA]), and an Agilent 1100 series auto-sampler (Agilent Technologies). The injected sample (100 µl) was pre-filtered via a 0.45-µm Chromafil® syringe filter (Macherey-Nagel, Germany) and then separated within a long channel (Wyatt Technology Europe) using a regenerated cellulose ultrafiltration membrane (nominal cutoff 10 kDa PLGC membrane; Millipore, Germany) and a spacer (350 µm high and 21.5 mm wide at its widest position). The injected sample was focused for 8 min by applying a focus flow of 4.0 ml/min. The cross-flow of the subsequent elution-steps was kept constant (4.0 ml/ min) for 5 min, and then it was decreased linearly to zero within two steps: (1) decrease to 0.2 ml/min within 10 min.
and (2) decrease to 0 ml/min within the next 10 min. The channel was rinsed for 21 min without an applied cross-flow [18].

Data were collected using ASTRA software v6.1.2 (Wyatt Technology Europe). The molar mass and molar mass distribution were calculated by Berry’s method using scattering angles of 57.0°–126.0°. The fractograms were divided into sub-fractions, which we had previously classified [18]: fraction 1 was assigned to (glycosylated) proteins [refractive index increment (dn/dc): 0.185 ml/g], fraction 2 was assigned to protein–polyphenol complexes (dn/dc: 0.146 ml/g), and fraction 3 was assigned to cell wall polysaccharides (dn/dc: 0.146 ml/g). Dextrins were spread over all fractions. The ratio of low: high molar mass polymer fraction was calculated by the quotient of dRI peak area fraction 1 divided by dRI peak area fraction 3 after their normalization to the total dRI peak area.

Sensory characterization of cereal-based beverages

The cereal-based beverages produced were sensorially characterized by the perception of the attribute of palate fullness and the specific mouthfeel descriptor of watery using a descriptive scheme with an intensity scale from 0 (not detectable) to 7 (very intense). Mouthfeel descriptors can additionally function as quality indicators of the superior attribute palate fullness [1]. Thus, the attribute watery was exemplarily chosen for monitoring, since it represents a specific descriptor of mouthfeel with a negative correlation to other descriptors of mouthfeel (e.g., body or viscous) [1].

A panel of 15 Deutsche Landwirtschafts-Gesellschaft e. V. (DLG)-certified panelists were chosen. The selected attributes of palate fullness and the mouthfeel descriptor watery were trained according to previous studies [1, 2]. Since the focus of this study was macromolecular profiling and their effect on the perception of palate fullness and mouthfeel, the panelists were instructed to wear nose clips to exclude volatile components. All beverages were sensorially evaluated in duplicate.

Statistical evaluation

Statistical analyses were performed using the JMP® Pro v13.1.0 software (SAS Institute Inc., USA). Normality was tested via the Shapiro–Wilk test (α > 0.05). The means of normally distributed data were compared using analysis of variance (ANOVA) and Tukey–Kramer HSD-test for post-hoc analysis. The means of non-normally distributed data were compared using the nonparametric Wilcoxon-test and Steel–Dwass-test for post-hoc analysis. Pearson’s correlation coefficient was used for correlation analysis.

Results and discussion

Malt composition

The analytical composition of the differently modified malts is shown in Table 1. By varying the malting parameters of the degree of steeping, germination temperature, and germination time, it was possible to produce differently modified malts with a wide range of malt quality parameters, especially for the macromolecular compounds. The degree of steeping mainly affected the proteolytic modification of grain malts. The soluble nitrogen ranged from 503 to 806 mg/100 g dry matter (conventional brewing specification [CBS]: 570–670 mg/100 g dry matter [33]), and FAN were set within a range of 65–229 mg/100 ml (brewing specification: > 140 mg/100 ml [33]). As expected, the malts produced from barley with increased crude protein content showed higher values for soluble nitrogen and FAN compared with barley with a lower crude protein content. The variation in germination temperature mainly affected the cytolytic modification of the malts. The concentration of β-glucan varied within 29–1370 mg/l (CBS: < 350 mg/l [33]). Accordingly, the viscosity of worts ranged within 1.401–2.618 mPa s (CBS: < 1.560 mPa s [33]), and the friability of the malts was adjusted within 53.7–98.7% (brewing specification: > 85% [33]). Hence, malts were produced that could be considered to range from under-modified within usual brewing specifications to over-modified. Generally, an increased crude protein content led to malts with a higher range of variation in their cytolytic modification. The extracts of all produced malts were comparable and did not show obvious variation in protein contents. Due to the high variations within malting parameters, some samples exceeded the defined quality specifications (specification values in brackets, according to Back et al. [33]), which assures brewing processability. However, since the objective was to study the influence of different malt modifications on the macromolecular profile and the perception of palate fullness, these extremes in malt modifications were required to gain a wide variety of compounds and macromolecular profiles.

Standard analytical parameters of cereal-based beverages depending on malting parameters

The standard analysis parameters of cereal-based beverages, which were produced from differently modified malts, are shown in Table 2. All beverages were produced identically and showed negligible variations in their original gravity (8.2–8.6 wt%) and in their pH (comparable per
Table 1 Standard parameters according to MEBAK of differently modified malts (variations in steeping degree, germination temperature, and germination time)

| Crude protein content (%) | Steeping degree (%) | Germination temperature (°C) | Germination time (days) | Friabilityb (%) | Extracta (% w/v) | Soluble nitrogena (mg/100 g dry matter) | FANa (mg/100 ml) | Viscositya (mPa s) | β-Glucana (mg/l) |
|---------------------------|---------------------|-------------------------------|-------------------------|-----------------|-----------------|------------------------------------------|----------------|-------------------|-----------------|
| Standard quality specifications for brewing to guarantee processability (isothermal 65 °C mash) | > 85 | > 81 | 570–670 | 130–160 | < 1.560 | < 200 |
| 9.9 | 42 | 12 | 5 | 69.6 | 76.2 | 503 | 65 | 1.864 | 1370 |
| | 42 | 12 | 7 | 87.5 | 76.3 | 579 | 105 | 1.607 | 739 |
| | 42 | 18 | 5 | 78.8 | 76.0 | 515 | 102 | 1.711 | 1252 |
| | 42 | 18 | 7 | 96.9 | 76.6 | 567 | 99 | 1.434 | 195 |
| | 48 | 12 | 5 | 82.4 | 76.4 | 552 | 71 | 1.673 | 939 |
| | 48 | 12 | 7 | 98.7 | 78.6 | 733 | 139 | 1.391 | 91 |
| | 48 | 18 | 5 | 88.6 | 76.5 | 606 | 121 | 1.538 | 455 |
| | 48 | 18 | 7 | 97.8 | 76.8 | 622 | 111 | 1.401 | 99 |
| 11.7 | 42 | 12 | 5 | 53.7 | 75.2 | 564 | 109 | 2.618 | 1370 |
| | 42 | 12 | 7 | 79.5 | 75.5 | 634 | 147 | 1.799 | 647 |
| | 42 | 18 | 5 | 73.1 | 76.1 | 537 | 110 | 2.095 | 1082 |
| | 42 | 18 | 7 | 89.3 | 76.6 | 595 | 98 | 1.492 | 271 |
| | 48 | 12 | 5 | 70.9 | 75.7 | 554 | 148 | 2.067 | 1058 |
| | 48 | 12 | 7 | 94.8 | 76.2 | 806 | 229 | 1.478 | 108 |
| | 48 | 18 | 5 | 85.4 | 75.6 | 607 | 127 | 1.684 | 441 |
| | 48 | 18 | 7 | 97.5 | 74.9 | 761 | 192 | 1.422 | 29 |

*aAnalyses performed after extraction according to isothermal 65 °C mashing procedure
bFriability was directly measured on malt kernels

Table 2 Standard analysis parameters according MEBAK of lactic acid-fermented cereal-based beverages produced from differently modified malts

| Crude protein content (%) | Steeping degree (%) | Germination temperature (°C) | Germination time (days) | Original gravity (wt%) | pH | Total-nitrogen (mg/l) | Viscosity (mPa s) | β-Glucan (mg/l) |
|---------------------------|---------------------|-------------------------------|-------------------------|-----------------------|----|----------------------|----------------|-----------------|
| 9.9 | 42 | 12 | 5 | 8.4 | 3.4 | 474 | 1.546 | 786 |
| | 42 | 12 | 7 | 8.4 | 3.4 | 528 | 1.462 | 249 |
| | 42 | 18 | 5 | 8.2 | 3.5 | 458 | 1.809 | 1062 |
| | 42 | 18 | 7 | 8.4 | 3.5 | 497 | 1.405 | 20 |
| | 48 | 12 | 5 | 8.5 | 3.4 | 517 | 1.572 | 618 |
| | 48 | 12 | 7 | 8.6 | 3.5 | 677 | 1.415 | 33 |
| | 48 | 18 | 5 | 8.3 | 3.4 | 548 | 1.412 | 169 |
| | 48 | 18 | 7 | 8.6 | 3.4 | 642 | 1.365 | < 15 |
| 11.7 | 42 | 12 | 5 | 8.4 | 3.7 | 488 | 2.041 | 1383 |
| | 42 | 12 | 7 | 8.4 | 3.8 | 577 | 1.590 | 620 |
| | 42 | 18 | 5 | 8.5 | 3.7 | 482 | 1.616 | 452 |
| | 42 | 18 | 7 | 8.5 | 3.7 | 612 | 1.500 | 217 |
| | 48 | 12 | 5 | 8.4 | 3.7 | 527 | 1.438 | 830 |
| | 48 | 12 | 7 | 8.6 | 3.7 | 780 | 1.340 | 118 |
| | 48 | 18 | 5 | 8.4 | 3.8 | 554 | 1.400 | 271 |
| | 48 | 18 | 7 | 8.5 | 3.7 | 737 | 1.303 | 81 |
experimental setup for sensory evaluation: pH 3.4/3.5 for 9.9% protein content and pH 3.7/3.8 for 11.7% protein content). However, a wide range of variation was successfully gained for macromolecular components due to differently modified malts (β-glucan, Δ 1302 mg/l and total nitrogen content, Δ 298 mg/l), as well as the resulting parameter viscosity (Δ 0.738 mPa s). During the brewing process of cereal-based beverages, the beverage composition is influenced by controlling the enzymatic depolymerization processes. These enzymatic reactions are technologically controlled during the malting and mashing process. Since mashing was conducted under constant isothermal conditions at a temperature that was chosen for the enzymatic degradation of starch (adapted standardized mashing procedure on demand of amylolysis are usual in practice), the present variations in beverage compositions were solely attributed to the different malt modifications. It can be assumed that the fermentation by L. amylolyticus did not affect the polymeric, unfermentable, and non-volatile composition of the beverages. Consequently, the non-volatile substance classes in the substrate and beverage (e.g., β-glucan) were almost equivalent and could be directly linked to the sensory perception of the intensity of palate fullness.

Each malting parameter affects the modification and the depolymerization of different substance classes of the malting barley (also dependent on the variety characteristics, protein content, provenience, and harvest year). Thus, because standardized substrate production procedures are common in practice, the malting parameters significantly influenced the final beverage composition. The viscosity and total nitrogen content differed significantly between different degrees of steeping. Additionally, gravity (ρ < 0.05, non-parametric comparison via Steel–Dwass), total nitrogen content, and β-glucan (both ρ < 0.01, nonparametric comparison via Steel–Dwass) differed significantly for variations in germination time. In general, a higher malt modification led to an increased total nitrogen content (increased proteolysis) and to lower viscosities and β-glucan-contents (increased cytolyis) in the final beverage. Thus, a wide range of variation in macromolecular components was successfully adjusted via the raw material characteristics.

**Macromolecular profile of cereal-based beverages measured by AF4/MALS/dRI**

The macromolecular profile of the produced cereal-based beverages was analyzed using AF4/MALS/dRI. Fractograms (dRI fractions 1–3) and the molar mass distributions are shown in Fig. 1 (MALS-signals were not shown, due to poor clarity). The corresponding macromolecular profile and molar masses are shown in Table 3. The indicated fractions represent the different substance classes, which we have previously classified within cereal-based beverages [18]. Differences in the elution behavior were monitored throughout all fractogram fractions for beverages produced from the differently modified malts. The samples produced from low modified malts showed higher signal intensities of dRI and dRI peak areas within all fractions since the high-molar-mass polymers were not depolymerized. Accordingly, samples produced with highly modified malts had lower signal intensities inside the fractograms and corresponding lower dRI peak areas throughout all fractions. In detail, an increasing tendency of cytolytic malt modification led to a decreased high-molar-mass polymer fraction (dRI fraction 3). Since the high-molar-mass polymer fraction consisted of cell wall polysaccharides, this decrease was in accordance with the decline of β-glucan concentrations for beverages produced with higher malt modifications. Correspondingly, an increase in proteolytic modification led to higher dRI signals within the low-molar-mass polymer fraction (dRI fraction 1), which was assigned to proteinaceous macromolecules [18]. However, the ongoing depolymerization of proteins was only detected for molar masses > 10 kDa, since molecules with a lower molar mass were not detected due to the cutoff of the channel’s ultrafiltration membrane.

The molar masses of all cereal-based beverages showed polydispersity and ranged from 10^3–10^8 g/mol. The molar masses (Table 3) and their distribution caused analogous effects to the dRI-signal’s elution behavior inside the fractograms due to the differences in the malts’ modification. Thus, the molar mass distribution was shifted to lower molar masses for beverages produced from malts with higher modification (increased cytolytic and proteolytic malt modification), since the macromolecules became increasingly depolymerized. However, a greater range of variation in molar mass distribution was observed for malts with increased crude protein content, which was consistent with a greater range of macromolecular compounds in the malts and beverages.

Since the malting profile definitely affected the molar mass distribution of cereal-based beverages, the influence of the respective malting parameters on the indices of the macromolecular profiles were further evaluated, revealing that the malting parameter germination time significantly influenced the number average molar mass (M_N), total dRI peak area, dRI peak area for fractions 2 and 3, and the ratio of low:high-molar-mass polymer fraction (ρ < 0.05, non-parametric comparison via Steel–Dwass). However, since the malting parameters affect the composition of the malt and, consequently, the composition of the beverage, significant correlations between total nitrogen and M_N (ρ < 0.1), total dRI peak area, dRI peak areas 2 and 3, and the ratio of low:high-molar-mass fraction (ρ < 0.05) were identified. Accordingly, the cytolytic analysis parameter β-glucan correlated significantly with M_N (ρ < 0.05), M_W (ρ < 0.05),
the total dRI peak area, the dRI peak areas of fractions 2 ($p < 0.01$) and 3 ($p < 0.001$), and the ratio of the low:high-molar-mass fraction ($p < 0.001$). Since gravity summarizes all dissolved compounds, a significant correlation of gravity on the total dRI peak area, dRI peak areas of fractions 2 and 3, and the ratio of low:high-molar-mass fraction ($p < 0.1$) were identified.

In summary, the macromolecular profile of the cereal-based beverages was affected by the differences in malt modification. Thus, the macromolecular profile in the final beverage can be technologically controlled by adjusting the raw material’s modification under generally applied and standardized substrate production conditions.

### Sensory characterization of palate fullness and mouthfeel

Table 4 presents the results of the sensory evaluation of the lactic acid-fermented cereal-based beverages, which were produced by different modified malts. Significant differences were found in the intensity of palate fullness and the mouthfeel descriptor watery (9.9% crude protein content) depending on the malt characteristics. As expected, samples produced from low modified malts showed the highest intensities for palate fullness. No significant differences in palate fullness were observed between different crude protein contents. However, the mouthfeel descriptor watery differed significantly between different crude proteins ($p < 0.01$, nonparametric comparison via Steel–Dwass).

### The link between sensory and analytical data

Significant correlations have been found between the perceived intensity of palate fullness, viscosity, and indices of the macromolecular profile (total dRI peak area and dRI peak area of fractions 1–3 (Table 5 and Fig. S1). In accordance with the lager beers [1], a correlation between the analytical parameter of viscosity and the intensity of palate fullness was observed, but with a lower correlation coefficient; however, the range of viscosity within commercial lager beers was narrower due to technological requirements and practicability. Although spiking experiments...
### Table 3: Means and standard deviation of molar masses and macromolecular profiles of cereal-based beverages from AF4-MALS-dRI (n=6)

| Crude protein content (%) | Steeping degree (%) | Germination temperature (°C) | Germination time (days) | \( M_N \) (kDa) | \( M_W \) (kDa) | Total dRI peak area (10^{-2} RIU min) | dRI peak area fraction 1 (10^{-2} RIU min) | dRI peak area fraction 2 (10^{-2} RIU min) | dRI peak area fraction 3 (10^{-2} RIU min) | Ratio low:high molar mass polymer fraction |
|--------------------------|---------------------|-----------------------------|------------------------|-----------------|-----------------|---------------------------------------|------------------------------------------|------------------------------------------|------------------------------------------|------------------------------------------|
| 9.9                      | 42                  | 12                          | 5                      | 44 ± 1.5        | 89 ± 6.5        | 7.89 ± 0.067                           | 2.54 ± 0.110                            | 1.83 ± 0.042                            | 3.52 ± 0.033                            | 0.722 ± 0.036                            |
|                          | 42                  | 12                          | 7                      | 37 ± 3.1        | 75 ± 2.2        | 4.44 ± 0.108                           | 2.38 ± 0.085                            | 1.20 ± 0.098                            | 0.86 ± 0.104                            | 2.795 ± 0.242                            |
|                          | 42                  | 18                          | 5                      | 39 ± 2.3        | 70 ± 7.9        | 6.58 ± 0.072                           | 2.44 ± 0.133                            | 2.07 ± 0.091                            | 2.07 ± 0.077                            | 1.181 ± 0.105                            |
|                          | 42                  | 18                          | 7                      | 37 ± 3.5        | 88 ± 20.0       | 3.70 ± 0.332                           | 2.48 ± 0.280                            | 0.91 ± 0.055                            | 0.31 ± 0.014                            | 7.966 ± 0.982                            |
|                          | 48                  | 12                          | 5                      | 40 ± 1.4        | 81 ± 8.0        | 6.58 ± 0.125                           | 2.66 ± 0.137                            | 1.93 ± 0.036                            | 1.99 ± 0.025                            | 1.335 ± 0.051                            |
|                          | 48                  | 12                          | 7                      | 36 ± 3.0        | 77 ± 10.0       | 3.73 ± 0.270                           | 2.52 ± 0.234                            | 0.89 ± 0.057                            | 0.33 ± 0.023                            | 7.821 ± 1.261                            |
|                          | 48                  | 18                          | 5                      | 36 ± 2.5        | 75 ± 8.1        | 4.48 ± 0.104                           | 2.56 ± 0.128                            | 1.24 ± 0.082                            | 0.68 ± 0.057                            | 3.798 ± 0.133                            |
|                          | 48                  | 18                          | 7                      | 40 ± 3.6        | 271 ± 60.7      | 3.04 ± 0.253                           | 2.07 ± 0.285                            | 0.72 ± 0.045                            | 0.25 ± 0.030                            | 8.539 ± 1.905                            |
| 11.7                     | 42                  | 12                          | 5                      | 57 ± 1.6        | 5723 ± 1622.6* | 6.70 ± 0.204                           | 2.34 ± 0.132                            | 1.35 ± 0.042                            | 3.01 ± 0.068                            | 0.778 ± 0.033                            |
|                          | 42                  | 12                          | 7                      | 38 ± 0.8        | 96 ± 25.2       | 3.96 ± 0.171                           | 1.86 ± 0.432                            | 1.13 ± 0.064                            | 0.97 ± 0.049                            | 2.559 ± 1.303                            |
|                          | 42                  | 18                          | 5                      | 37 ± 1.3        | 82 ± 11.0       | 3.20 ± 0.342                           | 1.27 ± 0.175                            | 0.91 ± 0.018                            | 0.95 ± 0.280                            | 1.454 ± 0.580                            |
|                          | 42                  | 18                          | 7                      | 34 ± 1.7        | 192 ± 123.5     | 2.65 ± 0.155                           | 1.50 ± 0.135                            | 0.83 ± 0.090                            | 0.32 ± 0.072                            | 4.936 ± 1.541                            |
|                          | 48                  | 12                          | 5                      | 45 ± 4.5        | 229 ± 161.0     | 4.04 ± 0.076                           | 1.39 ± 0.109                            | 1.00 ± 0.015                            | 1.65 ± 0.227                            | 0.859 ± 0.184                            |
|                          | 48                  | 12                          | 7                      | 36 ± 0.4        | 80 ± 5.2        | 2.37 ± 0.219                           | 1.52 ± 0.038                            | 0.64 ± 0.076                            | 0.22 ± 0.128                            | 5.396 ± 0.730                            |
|                          | 48                  | 18                          | 5                      | 37 ± 0.9        | 72 ± 2.5        | 3.05 ± 0.027                           | 1.37 ± 0.027                            | 1.02 ± 0.039                            | 0.67 ± 0.050                            | 2.063 ± 0.180                            |
|                          | 48                  | 18                          | 7                      | 34 ± 1.0        | 150 ± 97.8      | 2.99 ± 0.327                           | 1.73 ± 0.182                            | 0.95 ± 0.092                            | 0.31 ± 0.054                            | 5.712 ± 0.427                            |

*High standard deviation by variances in the high-molar-mass fraction of the distribution

\( M_N \) number average molar mass, \( M_W \) weight average molar mass
showed no correlation between palate fullness, mouthfeel, and the beverage’s viscosity in non-alcoholic beer [2], the indicated differences in the perception of mouthfeel [1, 2] could act as a quality descriptor of palate fullness [1]. Thus, the descriptor watery was significantly influenced by indices of the macromolecular profile (total dRI peak area and all sub-fractions within the dRI peak area (fractions 1–3) (Table 5). These findings confirm previous studies that investigated the effect of different macromolecular fractions on the perception of the intensity of palate fullness depending on the matrix composition by yeast-fermented cereal-based beverages (beer and non-alcoholic beer) and validated the effect of single substance groups (e.g., dextrins and β-glucan) by spiking experiments [1, 2].

In summary, the analytical tool AF4-MALS (macromolecular fractions) and the analysis of specific malt parameters provide a good indication of the sensory evaluation in terms of the intensity of palate fullness in cereal-based beverages.

### Conclusion

In recent years, the demand for cereal-based beverages has grown as a less sweetened alternative to classic non-alcoholic beverages. Market analyses have confirmed that the per capita consumption of non-alcoholic soft drinks (excluding water and fruit juices) has increased by 43% over the past 20 years in Germany [34]. Palate fullness and mouthfeel are key sensory attributes for consumer acceptance of cereal-based beverages; however, in contrast with the perceived harmony, both sensory attributes are often described as atypical and unbalanced. The perception of palate fullness and the mouthfeel can generally be influenced by the beverage’s composition and macromolecular profile.

We studied the influence of different raw material modification (as per cytolytic and proteolytic malt specifications) on the macromolecular profile and on the resulting sensory perception of palate fullness and the mouthfeel descriptor watery in cereal-based beverages. The beverages were produced from different modified barley malts using a standardized mashing procedure (production of the substrate), and the corresponding worts were fermented under standardized conditions using LAB. To achieve this, a strain of *L. amylolyticus* was selected for fermentation that did not produce exopolysaccharides. Thus, the macromolecular profile (the non-volatile composition of unfermentable substance classes) of wort remained constant throughout the fermentation process and was directly linked with the malt characteristics.

The different modified malts were produced by variation of the malting parameters of the degree of steeping, germination temperature, and germination time (Table 4).

### Table 4 Sensory evaluation (means) of the perceived intensity of palate fullness and mouthfeel descriptor watery of cereal-based beverages produced from differently modified malts (n = 15)

| Steeping degree (%) | Germination temperature (°C) | Germination time (days) | Palate fullness | Mouthfeel descriptor watery |
|---------------------|-----------------------------|------------------------|----------------|-----------------------------|
| 42                  | 12                          | 5                      | 4.50A          | 1.87B                      |
| 42                  | 12                          | 7                      | 3.71AB         | 2.74AB                     |
| 42                  | 18                          | 5                      | 4.25AB         | 2.25B                      |
| 42                  | 18                          | 7                      | 3.75AB         | 2.75B                      |
| 48                  | 12                          | 5                      | 3.79AB         | 2.08B                      |
| 48                  | 12                          | 7                      | 3.50AB         | 3.12B                      |
| 48                  | 18                          | 5                      | 3.37AB         | 2.91B                      |
| 48                  | 18                          | 7                      | 3.12B          | 2.78B                      |

Different letters in a column indicate significant differences (ANOVA and Tukey–Kramer HSD test: palate fullness: p < 0.05; watery: p < 0.1)

### Table 5 Correlation-coefficients (r) between sensory attributes and analytical data (n = 16)

| Analytical data | Palate fullness | Watery |
|-----------------|----------------|--------|
| Extract         | −0.2570        | 0.2161 |
| Total nitrogen  | −0.2699        | 0.2391 |
| β-Glucan        | 0.3735         | −0.2315|
| Viscosity       | 0.5363*        | −0.344 |
| M_N             | 0.1678         | −0.0132|
| M_W             | 0.1862         | 0.0474 |
| dRI peak area   | 0.6282**       | −0.6071**|
| dRI peak area fraction 1 | 0.5606*       | −0.5907**|
| dRI peak area fraction 2 | 0.5778*       | −0.6331**|
| dRI peak area fraction 3 | 0.5078*       | −0.4373 |
| Ratio low:high molar mass polymer fraction | −0.1522 | 0.0935 |

*p < 0.05, **p < 0.01, and ***p < 0.001*
germination temperature, and germination time, resulting in the concentrations of the specific malt substance classes (e.g., β-glucan, dextrins, and soluble nitrogen) covering a wide range of variance. Further, beverages prepared from differently modified malts showed a considerable variation in their beverage quality parameters, particularly in their macromolecular compounds.

The analytical method of AF4-MALS-dRI indicated differences in the macromolecular profile, molar mass and molar mass distribution of the produced cereal-based beverages. A higher malt modification led to decreased high-molar-mass fractions (depolymerization of cell wall polysaccharides) and an increased low-molar-mass polymer fraction (an increase of proteinaceous macromolecules by proteolytic modification). Accordingly, the molar masses and molar mass distribution were shifted toward lower molar masses. Malts produced from barley with increased crude protein contents showed a greater range within the macromolecular profile. The variation of germination time significantly influenced \( M_n \), the total dRI peak area, and the high-molar-mass fraction, which mainly contained cell wall polysaccharides (60–1200 kDa). Thus, the macromolecular profile was significantly affected by the malt’s modification.

The produced cereal-based beverages differed significantly in their sensory perception of the intensity of palate fullness and the specific mouthfeel descriptor watery. A higher malt modification led to a decrease of palate fullness and a corresponding increase of the mouthfeel descriptor watery (negative correlation). The perception of palate fullness was significantly correlated with different macromolecular fractions, such as proteins and cell wall substances, which were significantly influenced by the degree of steeping and resulting modification, respectively. The perception of the mouthfeel descriptor watery varied significantly for different crude protein contents; however, no significant differences between different crude protein contents were found for the perception of the intensity of palate fullness.

This study demonstrated that it was possible to control the perception of the intensity of palate fullness and the sensory attribute of mouthfeel by varying the malt modification, which offers a technological potential to modify the polymer distribution by the production of cereal-based beverages. However, the group of cereal-based beverages encompasses different types of beverages, which differ in the type of fermentation (e.g., using LAB). Since the fermentation did not affect the macromolecular composition, the results can easily be transferred to other cereal-based beverages (e.g., beer). The analytical tool AF4-MALS offers suitable applications for understanding how the macromolecular fractions in cereal-based beverages are linked to sensory evaluation. Thus, these results are beneficial for a targeted design of beverage composition, particularly the macromolecular profile, to influence the intensity of palate fullness by an improved selection of raw materials and adapted malting technology.

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Compliance with ethical standards

Conflict of interest The authors declare that they have 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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