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Chemometric modeling of palate fullness in lager beers

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ABSTRACT

Palate fullness and mouthfeel of beer are key attributes of sensory beer quality. Non-volatile substances and molar mass fractions influence sensory perceptions of palate fullness and mouthfeel. However, systematic correlations between sensory attributes and native beer compounds have not been evaluated within the concentration range found in lager beer. This article reports a chemometric analysis of 41 lager beers by evaluating analytical data of beer compositions, palate fullness, and mouthfeel descriptors. AF4-MALS-dRI indicated high variability in the macromolecular compositions of classical lager beers. Screened beers were clustered into groups differing significantly in *palate fullness* intensity and macromolecular distribution. Significant correlations were found between palate fullness and macromolecular fractions and beer composition parameters: original gravity, viscosity, indices of macromolecular distribution, total nitrogen ($p < 0.001$), and β-glucan ($p < 0.01$). Thus, a model was built using partial least square regression (PLS) analysis to predict the *palate fullness* intensity in beers ($R^2_C = 0.7993$). This model can be used as a guideline by brewers to control palate fullness and mouthfeel.

1. Introduction

Lager beer, a yeast-fermented cereal-based beverage (bottom fermentation), is a matrix including different volatile and non-volatile compounds. The general composition of lager beer consists of water, carbon dioxide (CO_2), alcohol, and extract which encompasses low molar mass compounds and different polymers. The technological important macromolecular fractions with molar masses up to 10^8 g mol^{-1} (Krebs, Becker, & Gastl, 2017) are classified into polysaccharides, proteins and protein–polyphenol complexes (Gresser, 2009). The polymeric profile of the final beer product depends on the raw materials and brewing technological parameters used (Choi, Zielke, Nilsson, & Lee, 2017; Rübsam, Becker, & Gastl, 2017; Rübsam, Gastl, & Becker, 2013; Wu, Du, Zhang, Ju, & Jin, 2015). Non-fermentable dextrins are derived from incomplete starch hydrolysis and their concentration varies with beer type and the mashing regime procedure (Rübsam, Gastl, & Becker, 2013; Rübsam, Krottenthaler, Gastl, & Becker, 2012). Proteins originate from raw materials. In addition, their concentration in beer is mainly determined by the raw material (raw material characteristics and proteolytic malt modification) and the mashing regime (Steiner, Gastl, & Becker, 2011). Furthermore, β-Glucan and arabinoxylan are fundamental cell-wall-polysaccharides of barley and their concentrations in beer are affected by the raw material (raw material characteristics and cytolytic malt modification) (Zielke, Teixeira, Ding, Cui, Nyman, & Nilsson, 2017) and the mashing procedure used (Kupetz, Procopio, Sacher, & Becker, 2015).

The use of asymmetric flow field-flow fractionation (AF4) has been shown to be an appropriate method for the separation of biopolymers. Absolute molar masses and their distributions can be determined by coupling AF4 with multi-angle light scattering detection (MALS), and concentrations are simultaneously measured by refractive index detection (dRI). Thus, the total polymer concentration and the relative amount of different macromolecular sub-fractions can also be determined (Nilsson, 2013; Podzimek, 2011; Wagner, Holzschuh, Traeger, Fahr, & Schubert, 2014; Yohannes, Jussila, Hartonen, & Riekkola, 2011).

AF4-MALS has been a field of active research for the characterization of macromolecular profiles, structural parameters, molar masses, and molar mass distribution in cereal-based beverages (Choi, Zielke, Nilsson, & Lee, 2017; Krebs, Becker, & Gastl, 2017; 2020; 2013; Krebs, Müller, Becker, & Gastl, 2018; Rübsam, Gastl, & Becker, 2012; Tügel, Runyon, Gómez Galindo, & Nilsson, 2015). It has been shown that molar masses and molar mass distribution of worts and beers can be technologically modified by using different raw materials, altering specific raw material characteristics and adjusting the malting regime or malting parameters (Krebs, Becker, & Gastl, 2020; Rübsam, Gastl, & Becker, 2013). Additionally, the mashing regime (control of enzymatic hydrolysis by temperature rests) can be changed due to the specific control of enzymatic depolymerization reactions (Choi, Zielke, Nilsson,

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& Lee, 2017; Rübsam, Becker, & Gastl, 2017). However, these findings obtained using AF4-MALS were based solely on the overall molar mass distribution, without consideration of individual substance classes or sub-fractions.

Therefore, based on a previously adapted AF4-MALS-fractogram to complex multi-component matrices in lager beers (Krebs, Becker, & Gastl, 2017), the authors extended the informative value of the established analytical method. To this end, different substance classes of beer polymers were assigned to respective molar mass fractions (peak area fractions). Proteins were assigned to low molar mass fractions (fraction 1). Middle molar mass fractions were attributed to protein–polyphenol-complexes (fraction 2) and the high molar mass fraction was assigned to cell wall polysaccharides (fraction 3). Dextrins were spread throughout all fractions (fractions 1 to 3) (Krebs, Becker, & Gastl, 2017). Using this more specific method it was possible to accurately attribute differences in molar mass distributions and molar mass profiles to different substance classes (Krebs, Becker, & Gastl, 2017). The application has already been successfully established to investigate different production methods of commercial non-alcoholic beers (Krebs, Müller, Becker, & Gastl, 2018). The sensory perception of palate fullness and mouthfeel was substantially influenced by the macromolecular components and molar mass distribution of different non-alcoholic beers (Krebs, Müller, Becker, & Gastl, 2018).

The sensory impression of a beverage is affected by the composition of volatile and non-volatile compounds in beer. Odorous volatile compounds reach the olfactory epithelium by two pathways: via the nostrils during sniffing and via the mouth during eating and drinking (Hummel & Seo, 2016). Non-volatile compounds are responsible for the sensation of taste, which involves the perception of one of the established basic tastes: sweetness, sourness, saltiness, bitterness, and umami. *Palate fullness*, *body*, *mouthfeel*, and *harmony* are sensory attributes for the characterization of beverages. These differ from the basic tastes but could be influenced by the non-volatile compounds of beverages (Krebs, Müller, Becker, & Gastl, 2018; Krebs, Becker, & Gastl, 2020; Langstaff & Lewis, 1993). Nevertheless, there is limited information on the characteristics and responsible substance classes, which influence the perception of *body*, *palate fullness*, and *mouthfeel* in cereal-based beverages. Since the focus of this study was macromolecular profiling, volatile components were excluded from the sensory tests by using nose clips (Krebs, Müller, Becker, & Gastl, 2018). This step is intended to isolate the link between chemical-analytical data and sensory perception related to specific molar mass fractions (low molar mass compounds, oligomers, and polymers) and the identification of palate fullness enhancing substance groups (Krebs, Müller, Becker, & Gastl, 2018).

The sensory attributes of *palate fullness*, *body* and *mouthfeel* are often used indiscriminately in literature because of missing or inaccurately defined classifications (Krebs, Müller, Becker, & Gastl, 2018). The definition of the attribute *body* includes descriptors such as consistency, compactness of texture, fullness, richness, flavor or substance of products (DIN EN ISO - 5492 Sensorische Analyse - Vokabular, 2009). The sensory attribute *palate fullness* was assessed in studies of different types of beverages including cereal-based beverages/beer (Krebs, Becker, & Gastl, 2020; Krebs, Müller, Becker, & Gastl, 2018; Rübsam, Gastl, & Becker, 2012) and other beverages (Gawel, Sluyter, & Waters, 2007; Guinard & Mazzucchelli, 1996; Guinard, Souchard, Picot, Rogeaux, & Sieffermann, 1998; Langstaff, Guinard, & Lewis, 1991; Langstaff & Lewis, 1993; Nurgel & Pickering, 2005; Parker, 2012; Vidal et al., 2004). Currently, an accurate definition of palate fullness and a clear distinction between *body* and *palate fullness* is absent in the literature. In conclusion, *palate fullness* is considered interchangeable with the term *body*. In contrast, the sensory attribute *mouthfeel* includes the mixed sensation of all chemical and physical impressions of a stimulus within the mouth (DIN EN ISO - 5492 Sensorische Analyse - Vokabular, 2009) and mouth-surface (Jowitt, 1974).

A pleasant perception of the sensory attribute *palate fullness* is crucial for a consumer's acceptance, and the drinkability of a beverage. Different substance classes have been studied for their influence on palate fullness, which differs according to their chemical structure and molar mass. Low molar mass components like glycerol, ethanol, polyphenols, and CO_2 are able to affect palate fullness (Langstaff & Lewis, 1993). Different polymers, such as dextrins, glycosylated-proteins, and β-glucans, were also shown to influence the perception of palate fullness (Langstaff, Guinard, & Lewis, 1991; Langstaff & Lewis, 1993; Lyly, Salmenkallio-Marttila, Suortti, Autio, Poutanen, & Lähteenmäki, 2004; Narziß, 2005; Ragot, Guinard, Shoemaker, & Lewis, 1989; Rübsam, Gastl, & Becker, 2012; Steiner, Gastl, & Becker, 2011; Wiesen, 2011). The effects of dextrins (Krebs, Müller, Becker, & Gastl, 2018; Rübsam, Gastl, & Becker, 2013) and β-glucans (Krebs, Müller, Becker, & Gastl, 2018) on the palate fullness and mouthfeel of commercial and non-alcoholic beers were previously studied. These effects were shown to be dependent on their molar mass.

The objective of this study was to evaluate factors that influence the perception of the intensity of *palate fullness* and selected descriptors of mouthfeel by using a holistic chemometric approach in commercial fresh lager beers. To this end, 41 commercial German lager beers with a wide range in beer compositions, macromolecular profiles (determined using AF4-MALS), specific beer quality parameters, and sensory perceptions of the intensity of *palate fullness* and mouthfeel were screened. The hypothesis of this study was that non-fermentable substance classes and their molar mass distribution are the main factors that affect the perception of palate fullness and mouthfeel. The chemometric approach is the basis for the modeling of palate fullness by the use of partial least squares regression (PLS) analysis as a function of beer composition and macromolecular parameters. This prediction model can be used for a targeted design of the intensity of *palate fullness* by adjusting of influencing factors (substance classes and molar mass fractions), which can be controlled by selecting the appropriate technological brewing parameters.

2. Materials and methods

2.1. Sample materials

Forty-one bottom-fermented commercial German lager beers were selected for the present study and bought at local markets at the metropolitan area of Munich. Brand names were omitted. The screened beers were classified into four main categories according to their original gravity and ethanol concentration: light beers (n = 10; gravity: 7.6 (wt%), alcohol (alc.).: 3.1 vol%), pilsner beers (n = 10; gravity: 11.4 (wt%), alc.: 5.0 vol%), German-style "Helles" / lager beers (n = 11; gravity: 11.6 (wt%), alc.: 5.1 vol%) and export beers (n = 10; gravity: 12.7 (wt%), alc.: 5.5 vol%). All beers were produced with 100% barley malt, fermented by bottom-fermenting lager yeast and brewed according to the German purity law. The carbonization was removed for the analytical applications by a 5 min ultrasonic degassing.

2.2. Beer composition analysis

The chemical composition was analyzed using standard procedures according to MEBAK-guidelines (Jacob, 2012): ethanol, original gravity, dynamic viscosity (measured by rotational viscometer, Stabinger, Anton Paar, Graz, Austria), total-nitrogen and β-glucan (R-110.43.174 (Jacob, 2016, 2013)).

2.3. Macromolecular characterization

The macromolecular profiles and molar masses were analyzed using asymmetrical flow field-flow fractionation (AF4) coupled with multi-angle light scattering (MALS) and diffractive refractive index detection (dRI), according to previously published methods (Krebs, Becker, & Gastl, 2017). In brief, the instrumental setup included an isocratic pump (Agilent 1100 series, Agilent Technologies, Germany), an automatic auto-sampler (Agilent 1100 series, Agilent Technologies, Waldbronn,

Germany), the AF4-instrument (Eclipse, Wyatt Technology Europe, Dernbach, Germany), a MALS-detection instrument (DAWN HELEOS, Wyatt Technology Europe, Dernbach, Germany) and a dRI-detection instrument (Agilent series 1260 RID VIS-LAMP, Agilent Technologies, Waldbronn, Germany). The separation was conducted within a separation channel (long channel, Wyatt Technology Europe, Dernbach, Germany) using an inserted spacer (350 μm high and 21.5 mm wide at its widest position) and a regenerated cellulose ultrafiltration membrane (nominal cutoff 10 kDa, Millipore, PLGC membrane, Darmstadt, Germany). Aliquots of 100 μl pre-filtered sample (0.45 μl, Chromafil®, Macherey-Nagel, Düren, Germany) were injected during an initial focusing period of 8 min (focus-flow: 4.0 ml/min). During elution (elution-flow: 1 ml/min), the initial cross flow (4 ml/min) was kept constant for 5 min and then was decreased linearly to zero in two steps. It was first decreased to 0.2 ml/min within 10 min and then it was decreased to 0 ml/min within the next 10 min. The channel was rinsed for 21 min without any cross flow. Eluent was 50 mM $NaNO_3$ and 0.025% NaN_3 and it is filtered by a 0.1 μm internally placed membrane filter (Supor, Pall Corporation, Port Washington, NY, USA).

Measurements were performed in triplicate and data were collected using ASTRA software (Wyatt Technology Europe, Dernbach, Germany: version 6.1.2). Fractograms were divided into three fractions, which were previously classified as glycosylated proteins (fraction 1; specific refractive index increment (dn/dc): 0.185 ml/g), protein–polyphenol complexes (fraction 2; dn/dc: 0.146 ml/g) and cell-wall polysaccharides (fraction 3; dn/dc: 0.146 ml/g). Dextrins were spread over all fractions. Molar masses were calculated using scattering angels within 57.0–126.0° using the Berry method. The ratio of low to high molar mass polymer fraction was calculated by the quotient of the dRI peak area of fraction 1 divided by the dRI peak area of fraction 3 after their normalization to the total dRI peak area.

2.4. Sensory characterization

All beers were characterized based on the sensory attributes of *palate fullness* and the attribute mouthfeel determined by specific descriptors. The intensity of *palate fullness* and selected mouthfeel descriptors (*watery*, *viscous/full-bodied*, *smooth/soft/creamy*, and *slimy*) was evaluated using an intensity score from 0 (not detectable) to 7 (very intense) according to a highly discriminating sensorial assessment scheme (Krebs, Müller, Becker, & Gastl, 2018). Mouthfeel descriptors can additionally function as quality indicators of the superior attribute *palate fullness*. Thus, the mouthfeel descriptor *watery* was exemplarily chosen as it represents the opposite term of *body* or *viscous*.

A panel was chosen of 13 DLG- (Deutsche Landwirtschafts-Gesellschaft e. V. (DLG, 2018)) certified panelists. All panelists were identically trained for these descriptors by spiked references, as previously shown (Krebs, Müller, Becker, & Gastl, 2018). Samples were placed in a temperature controlled lab 1 h prior to sensory evaluation to assure a sample temperature of 12 °C. All sensory evaluations were completed in duplicate.

2.5. Statistical evaluation

Statistical analyses were performed using the software SAS JMP® Pro 13.1.0 (SAS, Cary, NC, USA). Normality was tested via the Shapiro-Wilk-test ($p > 0.05$). Means of data that were not distributed normally were compared using the non-parametric Wilcoxon test and the Steel-Dwass test for posthoc analysis.

2.6. Chemometric evaluation

Chemometric analyses were performed using the software SAS JMP® Pro 13.1.0. Data were standardized to z-scores using auto-scaling before multivariate chemometric analysis. The Pearson correlation coefficient (R^2) was used for correlation analysis. Hierarchical cluster analysis (HCA) was used for the formation of different clusters, which showed similarities in their data. A PLS model was built for the prediction of palate fullness as a separated Y-variable. Therefore, the dataset was randomly split into a calibration set ($n = 30$) and validation set ($n = 11$) using data from all beer type categories before modelling. Models were built from the calibration data set only using factors, which correlated significantly to the sensory intensity perception of the attribute *palate fullness*. The number of latent variables was determined using leave-one-out cross-validation. Factors with scores of variable importance in projection (VIP scores) below 0.8 were excluded from the final model. After modelling, the model was validated using the independent validation data set. The model performance was evaluated by analyzing the percentage of variations in the x-/y-data, R^2, correlation coefficient of leave-one-out cross-validation (Q^2), the root mean square error (RMSEC) for the calibration data set, and the root mean square error of validation (RMSEV) for the cross-validation data set.

3. Results and discussion

3.1. Beer composition

The composition of the screened commercial beers is depicted in Table 1. Significant differences were verified for the means of alcohol content and original gravity between light beers, pilsner/lager beers and export beers ($p < 0.05$ Steel-Dwass-test). Since classical beer style categorization is based on gravity and ethanol, these differences were expected. The range of dynamic viscosity was 1.316–1.825 mPas, however, only the mean of light beers was significantly different compared to other beer styles. Thus, the viscosity range within commercial beer styles brewed by 100% barley malt is very narrow. The total nitrogen content varied from 36.5 to 96.4 mg/100 ml. Significant differences were found between light beers, pilsner/lager beers, and export beers ($p < 0.05$ Steel-Dwass-test). The concentration of β-glucan varied from < 15–452 mg/l. Significant differences were only found between light and export beers ($p < 0.05$ Steel-Dwass-test). Thus, this wide range of values is mainly dependent on the malt's cytolytic modification.

3.2. Macromolecular composition

Macromolecular indices (molar masses, total dRI peak areas and normalized ratios of low to high molar mass fraction) are shown in Table 1. AF4-fractograms and molar masses of representatives of each beer style are depicted in Fig. 1 A. The MALS-signals were not shown, and representatives of each category were randomly selected due to clarity. Fractograms were divided into different sub-fractions, which were previously classified (Krebs, Becker, & Gastl, 2017). The corresponding dRI peak areas of all screened beers are shown in Fig. 1 B. Molar masses of screened beers were distributed broadly within a range of 10^4–10^8 g mol^{-1}, which is consistent with previous studies (Choi, Zielke, Nilsson, & Lee, 2017; Krebs, Gastl, & Becker, 2016; Tügel, Runyon, Gómez Galindo, & Nilsson, 2015). In detail, the number average molar masses ranged from 34 to 54 kDa and no significant differences were found between different beer styles ($p > 0.05$ Steel-Dwass-test). The weight average molar mass widely ranged from 135 to 1532 kDa, however, only lager beers were significantly different from export beers ($p < 0.05$ Steel-Dwass-test). Thus, these wide ranges in molar mass indicate high variations within the molar mass distribution of traditionally classified beer styles.

Within the fractogram, light beer showed a lower dRI-signal throughout all fractions (Fig. 1A), likely due to their lower original gravity. In detail, the total dRI peak area of light beers differed significantly compared to export beers (Table 1, $p < 0.05$ Steel-Dwass-test) and all classified sub-fractions of light beers differed significantly compared to all other classical beer styles ($p < 0.05$ Steel-Dwass-test), except fraction 1 of pilsner beers. No significant differences in the peak

Table 1
The composition of screened beers according to MEBAK standard-analyses and macromolecular characterization using AF4-MALS-dRI.

Sample number[1]	Alcohol (vol%)	Original gravity (wt %)	Viscosity (mPas)	Total-N. (mg/100 ml)	β-Glucan (mg/l)	M_N (kDa)	M_w (kDa)	Total dRI-peak area (10^{-2} RIU min)	Ratio low to high molar mass polymer fraction
Style light beer (n = 10)	**3.0^{A} ± 0.32**	**7.6^{A} ± 0.30**	**1.37^{A} ± 0.042**	**50.6^{A} ± 11.22**	**150^{A} ± 63.1**	**40^{A} ± 4.1**	**530^{ABC} ± 356.2**	**3.33^{A} ± 0.642**	**1.61^{AB} ± 0.305**
1	3.2	7.5	1.32	46.8	138	42 ± 0.5	690 ± 107.1	2.90 ± 0.013	1.57 ± 0.013
2	2.5	7.4	1.38	53.4	186	39 ± 2.5	343 ± 18.6	3.65 ± 0.020	2.01 ± 0.013
3	3.3	7.8	1.37	46.5	199	39 ± 1.4	228 ± 44.6	3.26 ± 0.034	1.42 ± 0.026
4	2.4	8.3	1.47	78.7	273	45 ± 0.5	582 ± 81.8	2.95 ± 0.003	1.42 ± 0.015
5	2.9	7.2	1.36	43.6	141	37 ± 0.3	137 ± 5.4	2.85 ± 0.130	2.25 ± 0.010
6	3.2	7.5	1.34	36.5	71	47 ± 0.3	1349 ± 10.0	4.99 ± 0.028	1.30 ± 0.011
7	3.3	7.6	1.32	48.4	150	44 ± 0.2	762 ± 26.1	3.07 ± 0.027	1.40 ± 0.008
8	3.0	7.3	1.37	51.4	56	40 ± 0.1	577 ± 18.5	3.46 ± 0.005	1.38 ± 0.009
9	3.2	7.5	1.37	55.2	116	34 ± 0.3	228 ± 13.7	3.29 ± 0.040	1.68 ± 0.008
10	3.2	7.4	1.38	45.1	174	39 ± 1.9	408 ± 15.0	2.87 ± 0.009	1.65 ± 0.006
Style pilsener (n = 10)	**5.0^{B} ± 0.25**	**11.4^{B} ± 0.37**	**1.65^{B} ± 0.082**	**72.4^{BC} ± 7.04**	**241^{AB} ± 99.6**	**42^{A} ± 3.6**	**374^{ABC} ± 302.6**	**4.78^{B} ± 0.442**	**1.18^{BC} ± 0.3365**
11	5.6	12.2	1.63	77.3	323	46 ± 1.5	229 ± 13.3	4.52 ± 0.142	0.65 ± 0.050
12	5.1	11.2	1.66	66.5	192	48 ± 0.4	274 ± 8.8	4.20 ± 0.158	0.94 ± 0.090
13	4.9	11.1	1.61	69.8	260	39 ± 0.7	293 ± 15.1	5.27 ± 0.037	1.30 ± 0.021
14	5.0	11.7	1.58	74.1	< 15	40 ± 0.5	229 ± 3.7	3.92 ± 0.014	1.84 ± 0.021
15	5.0	11.4	1.62	87.6	283	46 ± 0.6	485 ± 5.8	5.12 ± 0.046	1.15 ± 0.004
16	4.9	11.1	1.68	63.8	239	42 ± 2.7	333 ± 12.7	4.81 ± 0.110	1.01 ± 0.029
17	4.9	11.3	1.58	76.2	365	37 ± 2.3	147 ± 11.0	5.39 ± 0.195	1.27 ± 0.021
18	4.7	11.7	1.78	73.1	260	40 ± 0.3	370 ± 5.1	5.20 ± 0.030	1.52 ± 0.063
19	4.8	11.1	1.58	70.0	190	39 ± 0.9	191 ± 12.1	4.73 ± 0.105	1.18 ± 0.042
20	4.8	11.1	1.81	65.2	291	43 ± 1.1	1190 ± 42.6	4.62 ± 0.115	0.91 ± 0.018
Style lager (n = 11)	**$5,1^{B}$ ± 0.16**	**11.6^{B} ± 0.18**	**1.68^{B} ± 0.067**	**73.5^{BC} ± 6.97**	**215^{AB} ± 69.0**	**41^{A} ± 2.6**	**341^{AB} ± 397.5**	**4.67^{B} ± 0.044**	**1.35^{ABC} ± 0.278**
21	5.0	11.4	1.77	68.7	257	39 ± 0.5	299 ± 2.0	5.09 ± 0.031	1.55 ± 0.152
22	5.2	11.5	1.63	67.8	282	43 ± 1.0	194 ± 1.9	4.48 ± 0.140	1.19 ± 0.060
23	5.2	11.6	1.63	64.5	308	38 ± 0.9	240 ± 13.0	4.93 ± 0.084	1.25 ± 0.009
24	5.2	11.7	1.62	77.8	116	42 ± 0.1	195 ± 1.1	4.40 ± 0.014	1.54 ± 0.006
25	4.9	11.7	1.69	75.9	254	46 ± 1.1	266 ± 3.1	4.12 ± 0.054	0.69 ± 0.505
26	5.2	11.6	1.67	73.5	122	40 ± 0.5	258 ± 1.7	4.41 ± 0.020	1.61 ± 0.003
27	4.9	11.5	1.63	73.4	273	41 ± 0.6	214 ± 26.5	4.45 ± 0.177	1.20 ± 0.040
28	5.2	12.1	1.83	81.3	259	43 ± 1.0	1532 ± 68.2	5.75 ± 0.117	1.24 ± 0.038
29	5.1	11.8	1.64	82.0	159	39 ± 0.1	211 ± 0.8	4.51 ± 0.040	1.40 ± 0.006
30	4.8	11.6	1.71	62.2	163	38 ± 0.3	208 ± 2.0	4.60 ± 0.030	1.54 ± 0.004
31	5.2	11.5	1.65	81.5	176	40 ± 1.6	135 ± 11.5	4.68 ± 0.072	1.640 ± 0.079
Style export (n = 10)	**5.5^{C} ± 0.25**	**12.7^{C} ± 0.43**	**1.61^{B} ± 0.051**	**83.3^{D} ± 7.78**	**257^{B} ± 87.1**	**44^{A} ± 4.1**	**613^{AC} ± 232.4**	**4.72^{B} ± 0.040**	**1.27^{BC} ± 0.1690**
32	5.8	12.5	1.57	75.5	314	39 ± 0.7	995 ± 25.4	4.29 ± 0.094	1.19 ± 0.019
33	5.4	13.0	1.67	87.7	156	47 ± 1.2	460 ± 11.4	4.65 ± 0.070	1.34 ± 0.990
34	5.6	13.0	1.66	82.7	223	42 ± 0.1	476 ± 22.3	4.88 ± 0.037	1.39 ± 0.018
35	5.3	13.3	1.67	96.4	452	40 ± 0.3	309 ± 27.0	4.69 ± 0.037	1.05 ± 0.005
36	5.5	12.2	1.53	82.4	192	47 ± 0.2	957 ± 52.4	4.57 ± 0.024	1.24 ± 0.029
37	5.0	12.2	1.61	77.4	185	43 ± 0.2	392 ± 5.60	5.04 ± 0.070	1.45 ± 0.004
38	5.8	12.3	1.55	83.1	205	53 ± 1.5	789 ± 18.2	5.61 ± 0.179	1.39 ± 0.018
39	5.2	12.2	1.61	70.9	312	45 ± 0.3	604 ± 38.7	4.26 ± 0.174	1.41 ± 0.018
40	5.6	13.0	1.60	93.5	255	43 ± 0.1	527 ± 9.2	4.49 ± 0.042	1.33 ± 0.006
41	5.4	13.1	1.66	83.8	278	43 ± 0.4	629 ± 29.0	4.71 ± 0.074	0.94 ± 0.011

1: The first row of each group shows the mean of the respective group ± standard deviation.
Different superscript letters in the same column indicate significant differences between groups ($p < 0.05$, nonparametric comparison according to Steel-Dwass-test).

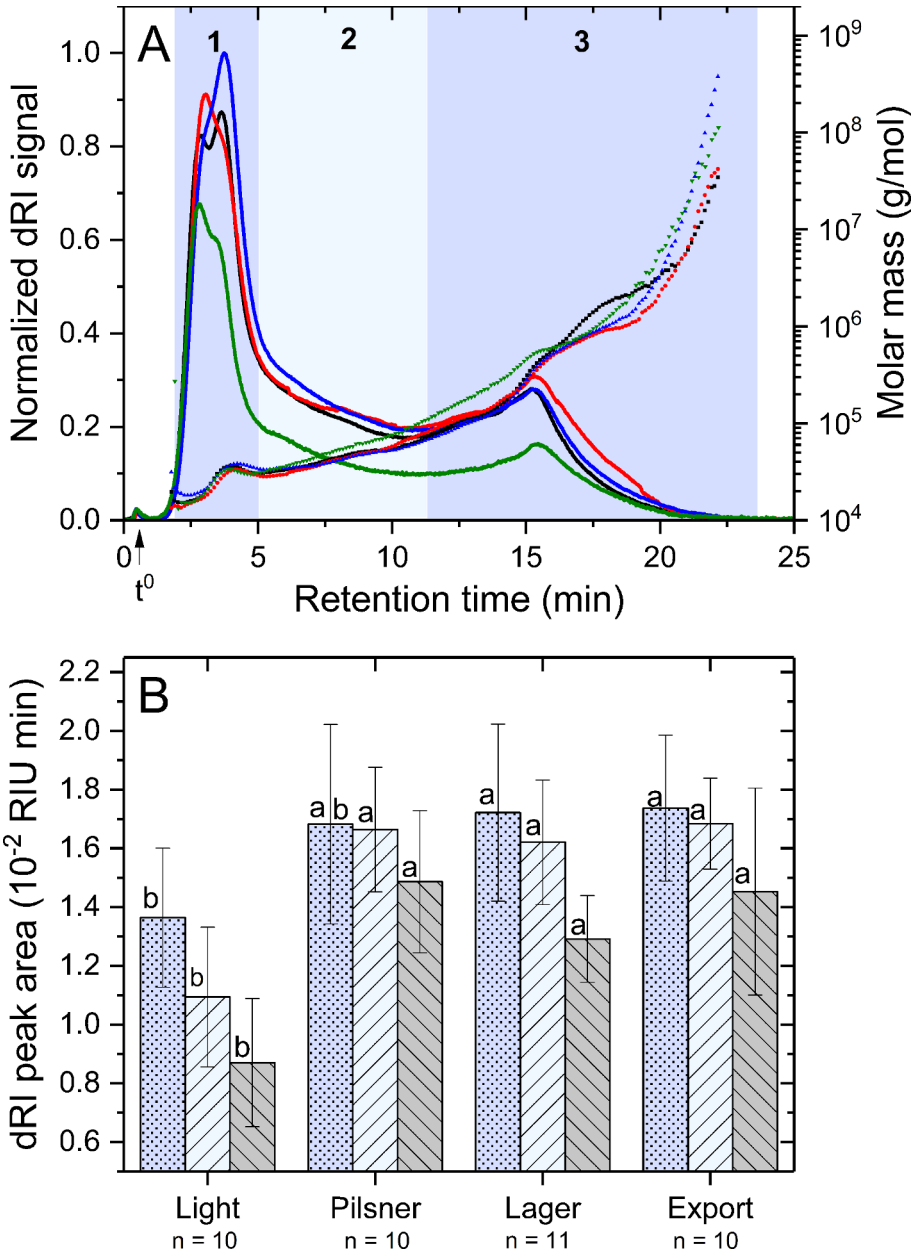

Fig. 1. A: Fractograms including the classified fractions of representatives of different beer styles (lines: normalized dRI signals, symbols: molar mass (g mol^{-1}), color description: green: light beer, red: pilsner, black: lager; blue: export). B: the mean of dRI peak areas of classified fractions (dotted bars: fraction 1, striped (right) bars: fraction 2, striped (left) bars: fraction 3). The different letters above the bars indicate significant differences ($p < 0.05$) according to nonparametric comparisons via the Steel-Dwass test. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

areas of the sub-fractions were found between pilsner, lager, and export beer types. However, high standard deviations of the means of the respective groups (Fig. 1 B) indicate high variability in macromolecular fractions within each classical beer style, which could be attributed to the different substance classes of macromolecules. Thus, the concentrations of proteins (fraction 1), protein–polyphenol-complexes (fraction 2), cell wall polysaccharides (fraction 3), and dextrins (fractions 1–3) were very different within each beer style, which is similar to the results obtained by standard composition analysis.

The macromolecular profile of beers is influenced by raw material modifications and mashing procedures (Choi, Zielke, Nilsson, & Lee, 2017; Rübsam, Becker, & Gastl, 2017; Rübsam, Gastl, & Becker, 2013). Thus, the present high variability within macromolecular fractions of classical beer styles are based on the diversity of available brewing technologies and raw material characteristics. Therefore, the influence of raw material (variety, provenience, modification) should not be avoided since mashing is industrially used for amylolytic degradation of starch and the crucial proteolytic and cytolytic degradation of polymers has shifted to the malting process, which precedes the brewing process (German purity law) (Wannenmacher, Gastl, & Becker, 2018).

Since the macromolecular profile of beverages affects the perception of the intensity of *palate fullness*, the present variations in the macromolecular profile of screened beers suggest high variability of intensity of *palate fullness* within the classical beer style range.

3.3. Sensory characterization

Boxplots of the sensory attribute *palate fullness* (A) and the representative mouthfeel descriptor *watery* (B) are shown in Fig. 2 for the screened classical beer categories. The means of *palate fullness* increased from light (2.95), lager (3.58) and pilsner (3.77) beers to export beers (4.59). Significant differences of *palate fullness* were found between

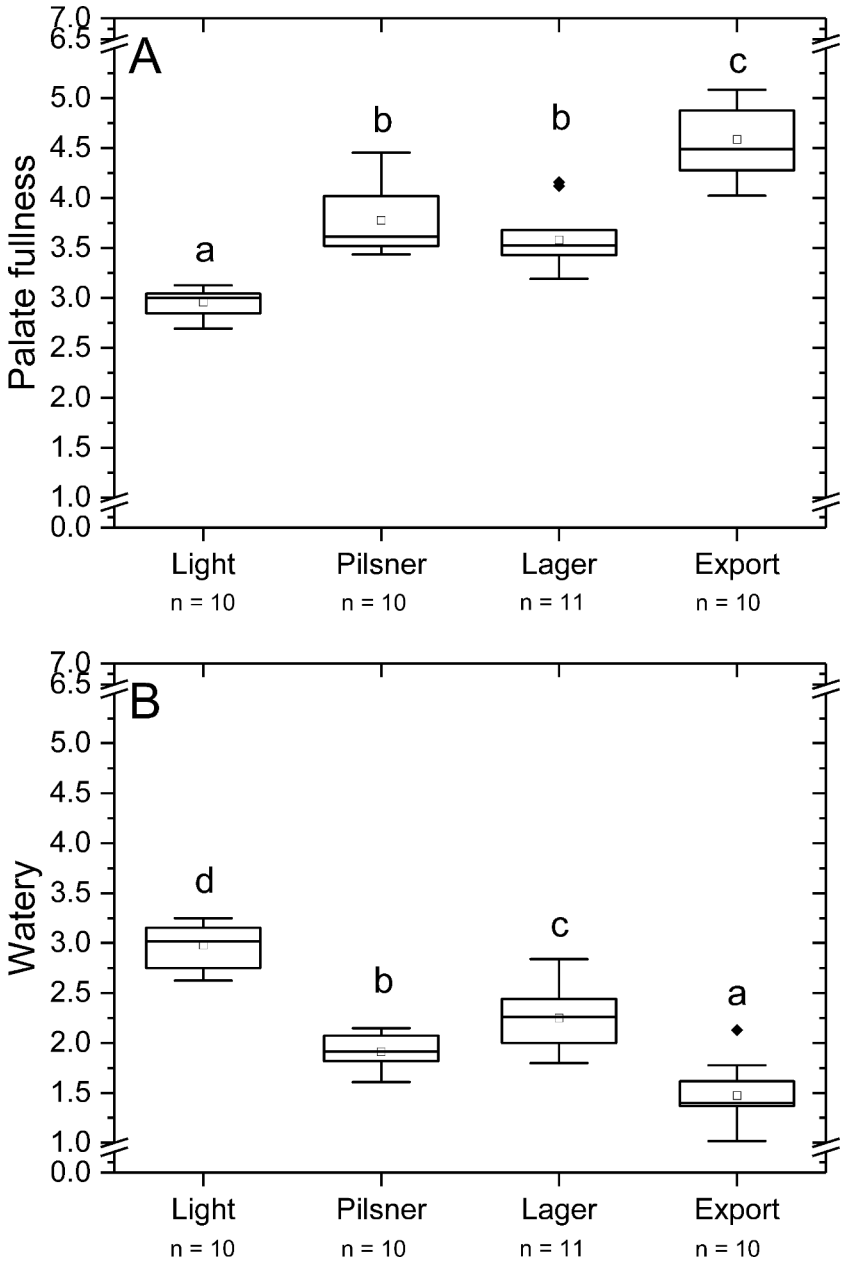

Fig. 2. The distribution of the sensory attributes palate fullness (A) and the mouthfeel descriptor watery (B) according to beer styles. Different letters indicate significant differences ($p < 0.05$) according to nonparametric comparisons via the Steel-Dwass-test.

light, pilsner/lager and export beers ($p < 0.05$ Steel-Dwass test for comparison of both individual scores of panelists and by the panelist's average by sample). No significant differences of *palate fullness* were found between pilsner and lager beers. The means of *watery* decreased from light (2.98), lager (2.25), and pilsner beers (1.90) to export beers (1.48). Significant differences of the mouthfeel descriptor *watery* were found between all beer categories ($p < 0.05$ Steel-Dwass-test). However, no significant differences were found for different beers within each category for both *palate fullness* and *watery* ($p < 0.05$ Steel-Dwass-test).

3.4. Chemometric approach

The objective of this study was to evaluate and model the influencing parameters of standard and macromolecular composition on the sensory perception of the attribute *palate fullness* and the specific descriptor of the attribute mouthfeel. Table 2 shows the correlation analysis of the sensorial attribute *palate fullness,* the descriptors of the attribute *mouthfeel*, and the analytical data (standard composition analysis and macromolecular indices). All evaluated attributes correlated significantly. Specifically, there was a positive correlation between *palate fullness* and mouthfeel descriptors, such as *viscous*, *full-bodied*, *smooth*, *soft*, *creamy*, *slimy*, and *sweet*, and a negative correlation between *palate fullness* and *watery*.

Significant correlations between *palate fullness* and the standard analytical parameters ethanol, original gravity, viscosity, total nitrogen content, and β-glucan concentration were found, which are consistent with previous studies. However, spiking studies of pure substances (polymers) in non-alcoholic beer showed no correlation between the analytical parameter viscosity and the intensity of *palate fullness* or the perception of mouthfeel (Krebs, Müller, Becker, & Gastl, 2018). Additionally, significant correlations were found between *palate fullness* and the number-average molar mass (M_N), the parameters of the macromolecular profile, such as the total dRI peak area, the dRI peak

Table 2
Correlation coefficients between the sensory evaluation and analytical data (n = 41).

	Palate fullness	*Mouthfeel descriptors*			
		Watery	Viscous, full-bodied	Smooth, soft, creamy	Slimy
Palate fullness	1.000***	−0.896***	0.925***	0.881***	0.648***
Watery	−0.896***	1.000***	−0.861***	−0.773***	−0.506***
Viscous, full-bodied	0.925***	−0.861***	1.000***	0.846***	0.541***
Smooth, soft, creamy	0.881***	−0.773***	0.846***	1.000***	0.737***
Slimy	0.648***	−0.506***	0.541***	0.737***	1.000***
Ethanol	0.742***	−0.796***	0.736***	0.553***	0.189
Original Gravity	0.808***	−0.852***	0.793***	0.623***	0.303
Viscosity	0.504***	−0.624***	0.533***	0.294	−0.040
M_N	0.316*	−0.311*	0.317*	0.408**	0.395*
M_w	−0.009	0.090	−0.092	0.069	0.305
Total dRI peak area	0.491**	−0.537***	0.508***	0.289	0.066
dRI peak area fraction 1	0.213	−0.311*	0.218	0.072	0.031
dRI peak area fraction 2	0.527***	−0.543***	0.552***	0.331*	0.131
dRI peak area fraction 3	0.533***	−0.589***	0.540***	0.373*	0.125
Ratio low-/high molar mass polymerfraction	−0.452***	0.430***	−0.458***	−0.370*	−0.134
Total nitrogen	0.722***	−0.766***	0.709***	0.565***	0.398**
β-Glucan	0.493**	−0.501***	0.549***	0.495***	0.252

* < 0.05.
** < 0.01.
*** < 0.001.

area of fraction 2, and the dRI peak area of fraction 3, and the ratio of low to high molar mass polymer fraction. Thus, palate fullness was significantly influenced by different substance classes, their individual concentrations (the range is corresponding with concentrations that are usually found in commercial German lager beers) and their polymeric profile (different molar mass fractions).

Palate fullness was chosen as a specific representative for further chemometric analysis because of the significant correlation between *palate fullness* and *mouthfeel* descriptors (Table 2). It supports the thesis, that the perception of mouthfeel can partly be considered as a quality description of palate fullness. Since classical beer styles showed high variations of analytical and macromolecular composition, a hierarchical cluster analysis (HCA) was conducted to evaluate the data and identify clusters with similarities (Fig. 3). Three clusters were identified, which are indicated by the different colors in the dendrogram. Cluster 1 (red color) included all light beers, cluster 2 (blue color) included most of the pilsner and lager beers and cluster 3 (green color) represented all the export beers. However, cluster 3 also included some pilsner and lager beers. The statistical evaluation of the clusters is shown in Table S1 using non-parametric comparisons via the Steel-Dwass-test. Significant differences within the clusters were found for the sensory attribute *palate fullness* and analytical parameters. The intensity of *palate fullness* within cluster 1 (2.95) was ranked ahead of clusters 3 (3.64) and 2 (4.24). As expected, the original gravity, ethanol-content, total nitrogen content, and β-glucan contents of cluster 1 (light beers) were significantly lower compared to clusters 2 and 3. However, significant differences in molar masses (M_N, M_W) were found between clusters 2 and 3, which differed from classical beer categorization. In agreement with previous studies (Krebs, Müller, Becker, & Gastl, 2018; Rübsam, Gastl, & Becker, 2013), the cluster with the highest number average molar mass (cluster 2) affected the highest palate fullness. As expected, significant differences were also found in the macromolecular profile between light beers (cluster 1) and the beers of clusters 2 and 3 due to their increased original gravity (cluster 2 and 3). Differences in the normalized ratio of low to high molar mass fractions were observed between clusters 2 and 3, which was previously shown to affect the sensory perception of palate fullness (Krebs, Müller, Becker, & Gastl, 2018). Thus, the clusters identified by HCA differed in palate fullness, which can be attributed to differences in their analytical and macromolecular compositions.

Since the influencing factors of palate fullness were assessed by correlation analysis (input parameters), a model for the evaluation of the intensity of *palate fullness* (output parameter) from the analytical data was built using a PLS analysis. It provides a tool for brewers to control palate fullness in natural beers. Therefore, the dataset was randomly split into a calibration data set (n = 30) and a validation data set (n = 11) using beers of each style. PLS analyses were conducted using leave-one-out cross-validation. Models of palate fullness were generated using analysis parameters, which correlated significantly to the impression of palate fullness and had VIP-scores above 0.8. Thus, a PLS model with four latent factors was chosen (Fig. 4). This model explained 89.9% of variations in the x-axis data and 79.9% of variations in y-axis data (R^2_C = 0.7993, Q^2 = 0.6464, RMSEC = 0.1321). The loading plot is shown in Fig. S1. This model was validated using the validation data set (R^2_V = 0.7538, RMSEV = 0.1025). As a result, palate fullness was modeled using the linear regression equitation:

$$\text{Palate fullness} = 1.078 \times A - 0.640 \times B + 0.059 \times C - 0.178 \times D + 0.055 \times E - 0.2981 \times F + 0.140 \times G - 0.008 \times H + 0.224$$

A: Original gravity
B: Viscosity
C: dRI peak area
D: dRI peak area fraction 2
E: dRI peak area fraction 3
F: Ratio low to high molar mass polymerfraction
G: Total nitrogen
H: β-Glucan

The original gravity was the highest influencing factor on palate fullness, which was expected since it correlates with the ethanol-content. In addition, the standard composition parameters viscosity, total nitrogen content, and β-glucan concentration affected the perception of palate fullness using this dataset. No molar masses were included in this model but fractogram fractions differentially affected the perception of palate fullness. This can be seen on the sign inside the equitation: total dRI peak area and the dRI peak area of fraction 2 (protein–polyphenol-complexes) and fraction 3 (cell wall polysaccharide). Thus, a molar mass depending effect of both the total macromolecular concentration and the high molar mass fraction (fraction 3) was found, which is consistent with the results of previous spiking studies of high molar mass fractions (β-glucan) (Krebs, Müller, Becker, & Gastl, 2018).

This prediction model can be used by brewers to influence the palate fullness in the final beverage. The standard analytical parameters,

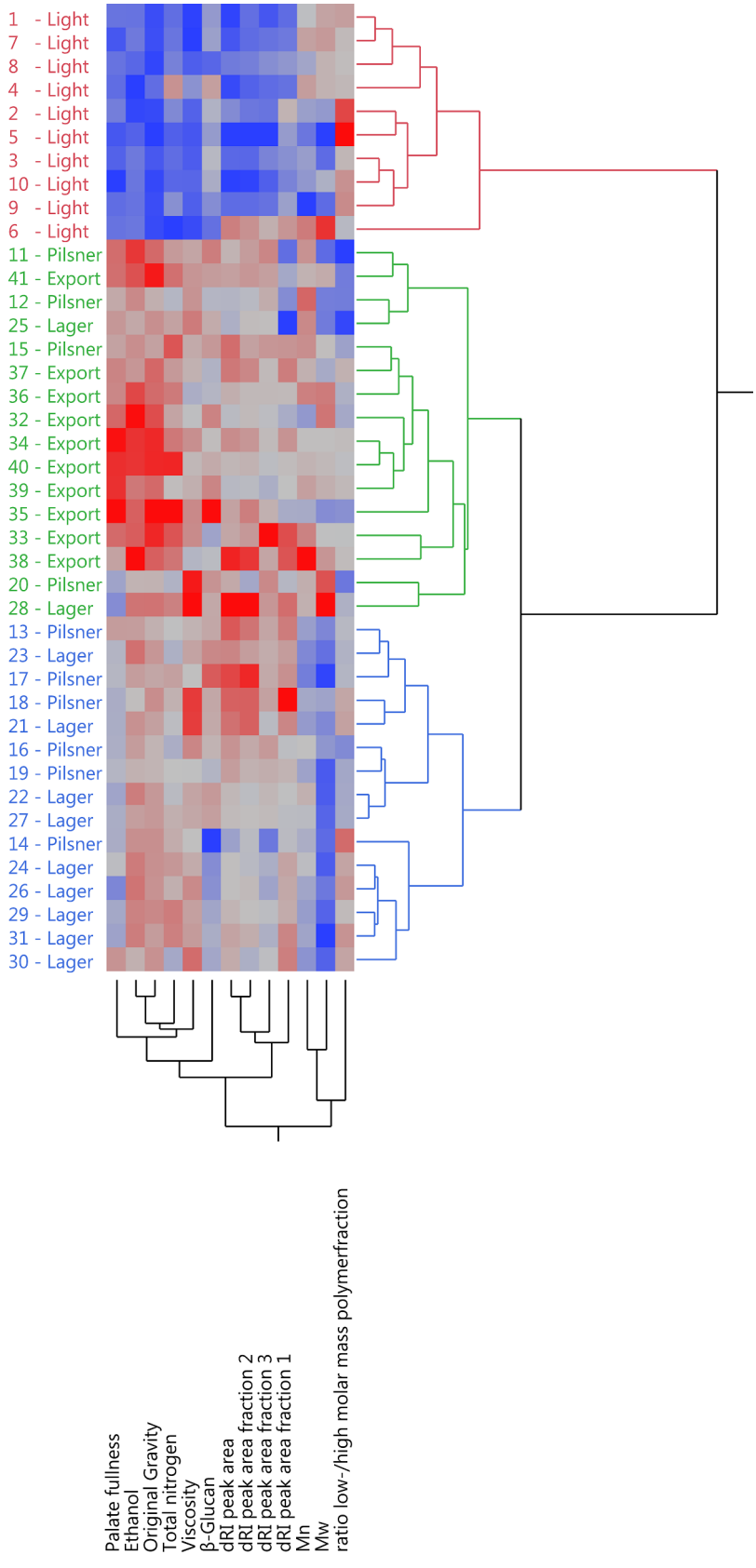

Fig. 3. The hierarchical cluster analysis of screened beers after data normalization. Different color intensities indicate individual values (red: 1 (maximum), blue: 0 (minimum)). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

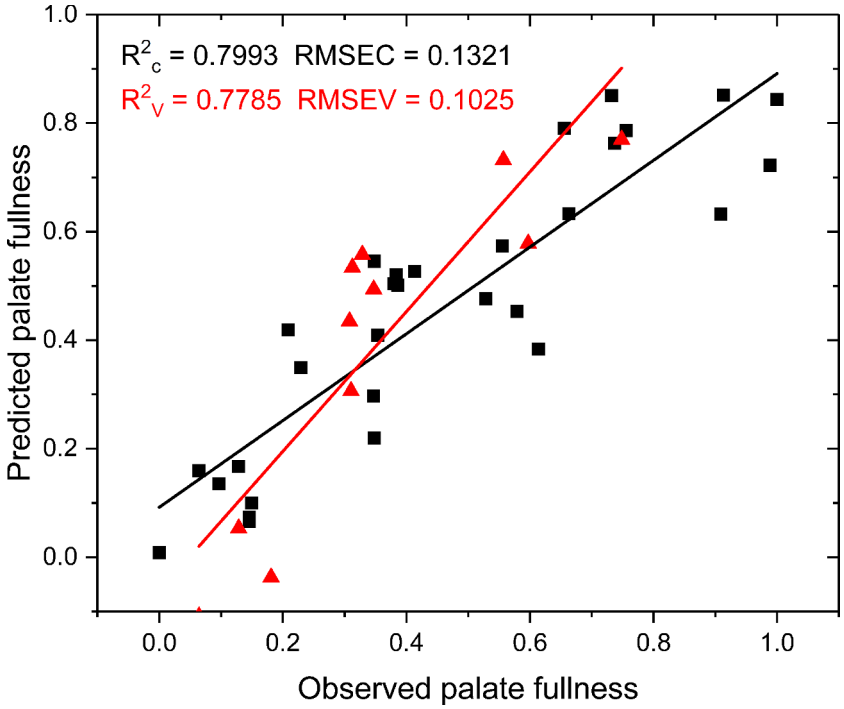

Fig. 4. Linear regression plot of the PLS model for palate fullness after data normalization. X-axis: observed values, y-axis: predicted values. The dataset was divided into calibration set (n = 30; black squares/line) and validation set (n = 11; red triangles/line). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

such as original gravity, total nitrogen, β-glucan content, and the macromolecular profile of the final beer are mainly technologically controlled via the malt's modification and characteristics (variety, provenience), mashing procedure, and degree of fermentation.

The holistic approach used in this study included standard composition analysis and analysis of the macromolecular profile via AF4/MALS/dRI. The results can be used to study and control the sensory perception of the sensory attributes *palate fullness* and *mouthfeel* of cereal-based beverages via the production process. In addition, this approach offers the possibility of demonstrating a link between analytical data and sensory evaluation.

4. Conclusion

The sensory perception of the attributes of *palate fullness* and *mouthfeel* are key factors that determine consumer's acceptance and quality of lager beer. The influencing factors on palate fullness and mouthfeel of commercial German lager beers were evaluated using a chemometric approach. High variability between different beer styles was found for their analytical standard composition and within molar masses and macromolecular fractions using AF4-MALS-dRI. Significant differences in *palate fullness* and the mouthfeel descriptor *watery* were found between different beer styles. Since the perception of palate fullness correlated significantly to all evaluated mouthfeel descriptors, *palate fullness* was chosen as a summary attribute for further chemometric analysis. Significant correlations were found between the perception of palate fullness and the analytical parameters ethanol, viscosity, number average molar masses, and indices of macromolecular profile. Hierarchical cluster analysis identified 3 clusters assigned to their analytical and macromolecular composition, which differed significantly in palate fullness. Partial least square regression was used for modeling the intensity of *palate fullness* from analytical data and to predict the sensory attribute *palate fullness* in beers by the analytical indices original gravity, viscosity, indices of macromolecular distribution, total nitrogen, and total β-glucan.

The prediction model can be used for a targeted design of palate fullness by the weighting of influencing factors, which can be controlled by technological brewing parameters and the selection of raw material characteristics.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.foodchem.2020.128253.

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