Doris Häge, Candy Ponickau and Bernd Lindemann

Sensory analysis and consumer acceptance of lager beer brewed with ancient spring barley (*Hordeum vulgare* L.) varieties

Given the limited understanding of the brewing performance and sensory characteristics of beer brewed with ancient barley (*Hordeum vulgare* L.) varieties, this study investigated lager beers brewed with Pilsner malt derived from ancient spring barley variety Mahndorfer Hanna, Kraffts Riedgerste, and Alpine Pfauengerste. For comparative purpose, modern spring barley variety Avalon was included in these small-scale brewing trials. Analytical parameters of the beers were determined, followed by descriptive sensory analysis. These results were compared with the previously determined sensory profile of hot steep malt extracts. At the end, a consumer acceptance test was conducted to see how consumers react to these beers. The findings indicate that the use of ancient spring barley varieties in brewing may have limitations for industrial-scale breweries, due to complications in solid-liquid separation during lautering. Lager beer brewed with ancient barley varieties showed significantly lower alcohol content 4.68 - 4.96 (% v/v) compared to Avalon (5.4 % v/v). Lager beer brewed with ancient spring barley variety Alpine Pfauengerste also differed significantly from all other varieties, for instance in terms of lowest apparent attenuation (74.1 %). Lager beers brewed with Mahndorfer Hanna and Kraffts Riedgerste exhibited in descriptive sensory analysis a significantly paler colour compared to beers brewed with Avalon, Results of beer analysis confirm these findings, as these two ancient spring barley varieties showed colour values of 5.8 EBC and 5.7 EBC. In contrast, lager beer brewed with the third ancient barley variety Alpine Pfauengerste was characterised by 11.3 EBC. In descriptive sensory analysis this lager beer was described as being significantly more brownish. It also exhibited a creamier, less watery mouthfeel. The sensory profiles of hot steep malt extracts were not predictive of the final beers' sensory profile. Consumer acceptance data revealed no significant differences in overall liking between beers brewed with Mahndorfer Hanna, Kraffts Riedgerste, and Avalon, whereas beer produced with Alpine Pfauengerste was significantly less liked. When brewing with ancient barley varieties, it is important to emphasise attributes such as local production and sustainability, which resonate strongly with consumers. Many are also willing to pay a premium for locally brewed beer made from ancient varieties. These ancient barley varieties may therefore hold particular value for craft beer brewers aiming to differentiate their products from standardised industrial beers.

Descriptors: Ancient spring barley, consumer liking, sensory descriptive analysis, craft beer, lager beer

1 Introduction

Ancient cereals are defined as "populations not subject to any modern breeding or selection, and sometimes retaining characters of wild ancestors, such as individual variability, height, brittle rachis, low harvest index and, in some taxa, hulled kernels" [1]. Up to now, there is no clear scientific way to differentiate modern cereal varieties from ancient ones, but often ancient cereal varieties are defined as being planted and processed before the start of the Green Revolution in the 1960s [2]. The impacts of climate change may also raise interest in cultivating these neglected cereals again. They might have specific traits like growing on marginal soil, which can be integrated into modern breeding programs [3]. Despite their lower agronomic performance, ancient barley (*Hordeum vulgare* L.) varieties might also be interesting for craft beer brewers, as they might bring specific flavour properties to the final beer [4]. Besides the term ancient barley [3], the expression heritage barley [5] is used in scientific literature. The use of ancient barley varieties for brewing might become more relevant as consumers nowadays search for new flavours in beer, as well as high-quality products [6], which leads to a still growing market share for craft beer [7]. The brewing industry underwent changes within the last few years. While the majority of beer is still standardised and brewed by large-scale industrial breweries, the craft beer industry is increasing [8]. Within the craft beer industry, beer is mainly brewed in small quantities, with local, high-quality ingredients [9]. The craft beer movement has generated a growing interest among consumers. Baiano (2021) stated that the most relevant differences between craft beer and commercial beer are the type of malt, hops, and yeast used, as

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Authors

Doris Häge (ORCID ID: 0009-0006-8775-8413), Bernd Lindemann, Department of Food Safety, University of Geisenheim, Von-Lade-Straβe 1, 65366 Geisenheim, Germany; Candy Ponickau, University of Geisenheim, Von-Lade-Straβe 1, 65366 Geisenheim; corresponding author: doris.haege@hs-gm.de

well as the application of non-standardised processing conditions and a cross of beer styles [10]. Craft beer brewers often tend to avoid pasteurisation and/or sterile filtration. Due to that, the final product has to be stored and distributed in a cold environment [11, 12]. How craft beer is defined varies between countries, but there are some legal definitions, established by national law, as well as working definitions given by regional or national trade organisations, for instance, the German Brewers Association or the US Brewers Association [10].

Despite a decrease in average beer consumption since 1990, due to a broader availability of other alcoholic and non-alcoholic beverages was well as changes in lifestyle and health concerns, beer is still one of the most consumed alcoholic beverages. Germans still consumed 88 litres of beer per capita in 2023. By contrast, the average per capita wine consumption in 2023 amounted to approximately 19 litres in Germany [13].

The raw materials used for beer brewing have a large influence on the beer's final sensory properties [14]. Malt, hops, and yeast are the main contributors to beer flavour [15], and the brewing process results in several hundred flavour-active compounds [16]. Beer contains a broad range of chemical components, which react and also interact at all stages of the brewing process [17]. Beer brewing relies on many different enzymatic reactions and microbial activity and consists of the following main steps: malt milling, mashing, lautering, wort boiling, and fermentation [14]. Milling of malt, mashing, and lautering are linked to each other due to the complex interactions and trade-offs arising between them [18]. The most important flavour-impacting stages are wort boiling and fermentation [15].

Beer quality and consumer acceptance are often determined by its flavour [19] as well as appearance [20, 21] and mouthfeel. The selection of appropriate attributes to describe the sensory properties of beer can be done with the help of an aroma wheel. The use of precise terms allows a detailed description of a beer's sensory profile in terms of aroma, flavour, and mouthfeel [23]. Beer consumption and choice are mainly affected by beer attributes and factors related to the purchase process [24].

Lager beer is the most important type of beer worldwide. It is fermented and matured at lower temperatures between 7 °C and 13 °C, leading to a very crisp and refreshing drinking experience [25]. The sensory profile of continental Pilsner beer can be described as being slightly sweet and grainy, with soft, slightly toasted, honey-like notes. It might be higher in dimethyl sulphide (DMS) precursors, which might result in a low corn-like DMS flavour in the final beer [26].

German lager beers are further characterised by an alcohol level of 3.5 to 4.5 w / w %, having, on average, a bitter substance content of 20 European Brewing Convention (EBC) bitterness units (BU). They also show a pH of 4.3–4.6 and typically measure between 8 - 15 EBC colour units. They are characterised by a viscosity of 1.4–1.7 mPas, as well as free amino nitrogen (FAN) content of 80–160 mg / L [27].

The sensory profile of lager beer tends to be smooth, clean, as well as free of esters. When stronger and darker lagers are considered, light ester notes are perceivable, which round out the flavour. In contrast, pale lager, especially fresh ones, might show some weak but pleasant sulphur notes which originate from yeast. Some sulphur notes might be fleeting, which is not considered as an off-note, whereas an aroma of rotten egg or sewer gas is considered as a fault [26]. Bettenhausen et al. (2020) identified alkane/alkenes, benzenoids, amides/amines, and fatty acid esters as volatile compounds relevant for the most desirable lager flavour. On the contrary, Maillard reaction products led to lager beer with a less preferred sensory profile. Lager beer disliked by consumers showed attributes like molasses, bitter, toasted, and malty [28]. The same holds for lager beer with an intense cabbage and cheese-like aroma [29]. According to Schieberle (1991), 3-methylbutanol (malty), 2-phenyl-ethanol (flowery, honey-like), 3- and 2-methylbutanoic acid (sweaty, rancid), 4-vinyl-2-methoxy-phenol (spicy, clove-like), furaneol, ethyl-butanoate (fruity), (E)-β-damascenone (cooked-apple), sotolone (hydrolized protein) and butanoic acid (buttery, rancid) are the most important odorants in pale lager beer [30].

Difference from Control (DFC) test is a sensory test method widely applied in quality control. It can be described as a multiple sample difference test. The aim is to assess, if a difference exists between one or more samples, in comparison to a control sample. It also determines the magnitude of difference between the control and the samples tested with the help of a difference scale. Often, blind samples are incorporated to test the panel's internal validity by assessing the control against itself, providing a baseline when comparing the control with other samples tested [31, 32]. Leong et al. (2017) studied the effect of sodium reduction and replacement with flavour enhancers in typical Asian food using DFC test [33]. This type of test is very applicable for heterogeneous types of products, as Aust et al. (1985) verified with various products from pizza to beef broth [34]. Schumaker et al. (2017) even applied DFC test with consumers to test consumers' ability to detect Brettanomyces volatile aroma compounds in red wine. Showing that consumers were able to perceive differences in red wine aroma due to ethylphenols formed by a common spoilage yeast, *Brettanomyces buxellensis* [35].

In sensory analysis, descriptive methods are used to describe a product in a qualitative and quantitative manner. A product's characteristics are defined, and the intensity of each attribute is assessed. The result will be an individual profile of the respective product tested. These product profiles can be used to characterise and differentiate products from each other. When interlinked with hedonic data, descriptive analysis will help to understand which attributes might be the reason why a specific product is liked or disliked by consumers. Sensory profiling consists of different phases. At the beginning panellists undergo product-specific training and training on the correct use of the scale, before the actual measurement phase on intensity scales [36]. Descriptive analysis can be applied to a wide range of food and beverages. Laureati et al. (2012) used sensory profiling to determine if celiac and non-celiac subjects describe gluten-free bread in a different way [37]. Descriptive analysis, among other methods, was also successfully applied for the description of commercial green table olives [38]. Descriptive analysis was also utilised to study the sensory profile of wholegrain buckwheat-enriched pasta [39] or traditional Italian pasta filata cheese [40]. Descriptive analysis can also be used to characterise different beer styles [29] or to show the difference in the sensory profile of commercial alcoholic and non-alcoholic Pilsner beers [41]. It is also a useful method to determine the sensory characteristics of craft beers [42].

Assessing acceptability, preference, and purchase intention are some of the traditional quantitative studies widely used in the food and beverage industry [43]. For hedonic tests, often a 9-point hedonic scale is presented to consumers. It is considered as a liking scale used to measure preference. There are different versions available. On the one hand, the 'words only' version, where each number from 1 to 9 is assigned to a word for statistical analysis. On the other hand, the numerical 'numbers only' scale, which is labelled at the ends with 1 = dislike the most, and 9 = like the most, and sometimes at the midpoint 5 = neither like nor dislike [44]. Additionally, aspects like consumers' purchase intention can be determined, for instance, by using a 5-point structured scale labelled as follows: 1 = certainly would not buy and 5 = certainly would buy [39, 45]. Consumers might also be asked about their willingness to pay by asking, for instance, "How much would you spend for a 0.5 L bottle of the beer tested?", followed by price categories where consumers are able to choose from [46]. Hedonic tests can also contain socio-demographic data, for instance, age, gender or frequency of consumption of specific products [39]. Hedonic tests are applied to a variety of products and in different contexts. For instance, Laureati et al. (2012) compared the sensory and hedonic perception of gluten-free bread by celiac and non-celiac consumers, to see if the two groups might differ in their preference [37]. Stokes et al. (2017) conducted sensory acceptance testing with instant and filtered coffee of the most popular coffee brands on the Irish and UK market [47]. Yilmaz and Aydeniz (2012) studied how differences in the sensory characteristics of green table olives affected consumer preference and buying intentions [38]. Esposito et al. (2014) studied consumer liking of typical Italian pasta filata cheese [40]. Craine et al. (2021) asked consumers to assess beer samples of different barley genotypes using a 9-point hedonic scale [48]. Cela et al. (2023) used hedonic tests to study the influence of the main brewing techniques for gluten-free beer with unconventional gluten-free cereals on consumer liking, willingness to buy and willingness to pay [46]. Consumer reactions to different beer styles can also be determined by hedonic tests. It might also be able to assess which attributes are relevant for consumers to like or dislike a specific beer style or type of beer [29, 41].

The "Hot Steep Malt Sensory Evaluation Method" (HSME) is a quick and easy method, which enables the assessment of the brewing malt sensory profile in a quick and reproducible manner [49]. The preparation of the so-called hot steep malt extracts takes about 30 minutes and reduces conversion time and temperature. So the malt's flavour compounds can be perceived without the intense sweetness of a congress wort. This method is also favourable if only a small quantity of brewing malt is available [50]. HSME was validated and approved by the American Society of Brewing Chemists Sensory Technical Subcommittee (ASBC) [51]. Further research is needed to investigate if HSME is suitable for predicting the aroma and flavour profile of resulting beers [50].

The Check-All-That-Apply (CATA) Method is considered a rapid, frequency-based sensory profiling method. It is a valuable method for the sensory characterisation of food products. CATA can be conducted with trained panellists as well as untrained consumers. Sensory assessors are provided with a list of attributes relevant to describe the products tested and are supposed to mark attributes that are most relevant to characterise the tested product. The results show how often an attribute is used by the assessors to describe the product. The more often an attribute is selected, the more relevant it is to describe the product [52].

So far, little is known about the sensory properties of beer brewed with malt of ancient spring barley (*Hordeum vulgare* L.) varieties. Also, not much knowledge is available about how consumers react towards beer brewed with ancient spring barley varieties. The aim of this study is to provide a sensory description of lager beer brewed with ancient spring barley varieties (Mahndorfer Hanna, Alpine Pfauengerste, Kraffts Riedgerste) in comparison to a modern bred spring barley variety, Avalon. Further consumer preference was investigated together with consumers' willingness to buy and willingness to pay for lager beer brewed with Pilsner malt from ancient spring barley varieties. Finally, this research aims to determine if the sensory profile of hot steep malt extracts found by Häge et al. (2025) [53] can be transferred into the final beer.

2 Material and methods

2.1 Brewing process

Lager beers were brewed using Pilsner malt from ancient spring barley varieties Mahndorfer Hanna, Kraffts Riedgerste, and Alpine Pfauengerste, vintage 2020. For comparison, Pilsner malt from the modern spring barley variety Avalon, vintage 2020, was added, too. All samples were grown in the Lower Rhine region in North Rhine-Westphalia. An Avalon industry standard (Durst Malz - Heinrich Durst Malzfabriken GmbH & Co. KG, Bruchsal, Germany, vintage 2020) was incorporated, too. Malting was carried out as a, for all varieties standardised, modified Central European Commission for Brewing Analysis (MEBAK) small malting process using a rotating germination box. Steeping times were observed according to the regulations, but pre-sorting of the barley was omitted. A kilning programme for Pilsner malt was used, as the aim was to keep the spring barley varieties' characteristic flavour by using lower kilning temperatures. Malting parameters were kept the same for all barley varieties and were not adjusted to each variety. Malting as well as the brewing process took place at the University of Geisenheim, Institute of Beverage Technology. Complete results of malt analysis following MEBAK standards, are presented in Häge et al. (2025), but most relevant details can be summarised as follows: Pilsner malt from modern barley variety Avalon was mainly within the MEBAK reference values for ideal pale malt. The raw protein content of the ancient spring barley varieties ranged between 12.9 % and 17.1 %. Especially ancient spring barley variety Alpine Pfauengerste exceeds the ideal raw protein content of malting barley, with 17.1 %. This variety also had with 82.5 % the lowest amount of malt of 1st grade. The other ancient spring barley varieties Kraffts Riedgerste and Mahndorfer Hanna were with 86.7 % and 90.6 % below the MEBAK minimum requirements for malt of 1st grade, too. All malt samples from the ancient barley varieties showed lower friability values i.e. below the MEBAK reference value. Alpine Pfauengerste had a friability value of 40.2 %, Mahndorfer Hanna of 40.5 % and Kraffts Riedgerste of 49.4 %. Results of congress mashing procedure showed slow filtration rates for ancient barley varieties Mahndorfer Hanna and Kraffts Riedgerste, while filtration of Alpine Pfauengerste was stopped after 120 min. The lowest extract value was determined for malt from Alpine Pfauengerste (67.0 g/100g in dry matter), the other two ancient spring barley varieties Kraffts Riedgerste and Mahndorfer Hanna showed extract values of 72.2 (g/100g in dry matter) and 72.3 (g/100g in dry matter) [53].

Infusion Mashing started with 5 kg of conditioned malt grist, which was added at 45 °C to 20 L of demineralised water. Where required, the pH-value of the mash was adjusted with 80 % lactic acid to 5.5 pH-value. First rest (proteases rest) took place at 52 °C for 20 min. Second rest (maltoses rest) for 20 min. at 62 °C. Third rest (saccharification rest) at 72 °C for 20 min. Shortly before reaching the mashing off temperature of 78 °C, a sample was taken to test for iodine neutrality. Afterwards, the mash was transferred from the mashing tun to the lauter tun. Lautering rest was 15 min. The first litre of turbid run-off was transferred back to the lauter tun. Sparge water (tap water) was given at 78 °C to reach 12 Brix of sweet wort.

Wort boiling took place for 60 min., with low hop dosage (12 g Hallertau Herkules, pellets 90, alpha-acid content 16.8 %, Hopsteiner, Mainburg, Germany) to keep the barley varieties' sensory profile. Hop was added 10 minutes after the start of wort boiling, followed by a 30 min. whirlpool rest. When required, the Brix value was adjusted to 12 ° Brix by adding tap water to reach an original gravity of 11.5 °Plato. Afterwards, the wort was pumped into the fermentation tank after a two-stage wort cooling process. For fermentation, 30 g bottom-fermented yeast (*Saccharomyces carlsbergensis*, BrewMasters German Classic W34/70 3G, Erbslöh, Geisenheim, Germany) was added at 20 °C to the wort in the fermentation tank. Fermentation took place at 10 °C for one week, for a week at 15 °C, and for three days at 4 °C. Yeast was removed afterwards. Beer was not filtered and bottle fermented in brown 0.33 L bottles by the addition of 2.3 g of sucrose per bottle. Bottles were afterwards closed with bottle caps and left for a second fermentation for 14 days at 20 °C and for 21 days at 5 °C. Afterwards, samples were stored at 10 °C until sensory tasting. Due to the adjustment of mash pH with lactic acid and the addition of sucrose for a second fermentation, lager beer was not according to the German Purity Law.

2.2 Chemical and physical analysis

Analysis of brewing water was done in duplicate following MEBAK [54]. Determining total hardness (GH), carbonate hardness (CH), and non-carbonate hardness (NCH), all in degree of hardness (°dH). Residual alkalinity (RA) was calculated according to Staudt (2015) [55] using the following formula:

RA (° dH) = KH - (GH/4)

Spectrophotometric colour analysis and determination of free amino acid (FAN) content took place according to MEBAK [56], with spectrophotometer DR 6000 (Hach Lange GmbH, Düsseldorf, Germany) at 430 nm. Determination of FAN content was done in triplicate.

Analysis of bitter units (BU) was done with cuvettes-test LCK 241, HACH, and spectrophotometer DR 6000 (Hach Lange GmbH, Düsseldorf, Germany).

Alcohol content, original gravity, apparent attenuation and extract content were determined with densitometer DMA 4500 and Alcolyzer Beer (Anthon Paar GmbH, Ostfildern-Scharnhausen, Germany). Analysis of pH-value was carried out using pH measuring device Five Easy (Mettler-Toledo GmbH, Berlin, Germany). All measures, with the exception of bitter units and FAN, were conducted in duplicate. Analysis of Variance (ANOVA) and Tuckey post-hoc test at confidence level of 95 % were carried out using XLSTAT.

2.3 Sensory analysis

DFC test took place at the sensory lab, Department of Enology, University of Geisenheim, equipped according to ISO 8589 [57].

Samples were tasted by 15 sensory trained panellists in three replicates. Panellists underwent basic sensory training according to ISO 8586 [58] and were familiar with the sensory properties of beer, as well as with the execution of DFC tests. Beer was served at 10 °C and with an amount of 50 mL, to panellists in transparent ISO 3591 wine tasting glasses [59] in randomised order, coded with three-digit codes. Water and unsalted bread (matzo) were provided to panellists as palate cleansers.

DFC test was conducted using a 6-point scale from "no difference to the reference sample" to "very large difference to the reference sample". Differences were assessed for the modalities appearance, aroma, flavour, mouthfeel, and aftertaste separately. In addition, a free comment field was provided where panellists could describe the perceived difference, if needed. Each panellist received a beer aroma wheel [29] for a standardised description of differences. As a reference lager beer brewed with Avalon industry standard was provided. The reference was also incorporated in the sample set.

Data was collected computer-based, ANOVA and post-hoc Dunnett Test were applied for statistical analysis, using FIZZ package 3.7, FIZZ Biosystems, Courtenon, France.

Descriptive Analysis of beer samples was done by following ISO 13299 [60] and took place under the same conditions as the previous DFC test, with the same panellists who were familiar with descriptive profiling of beer, too. Attributes relevant to describe lager beer [23, 61] (see Tab. S1) were assessed on unstructured line scales, which were labelled at the beginning and the end of the scale. The attribute list comprised 31 descriptors in total for the modalities appearance, aroma, flavour, mouthfeel, and aftertaste. Statistical analysis included ANOVA and post-hoc Tukey Test at 95 % confidence level. For data collection as well as statistical analysis, FIZZ package 3.7, FIZZ Biosystems, Courtenon, France, was used.

2.4 Consumer acceptance test

Before the tasting session, participants had to confirm that they had reached legal drinking age (16 years in Germany) and that they consume beer regularly, i.e., at least once per month. Participants had to confirm that they do not suffer from allergies or food intoler-

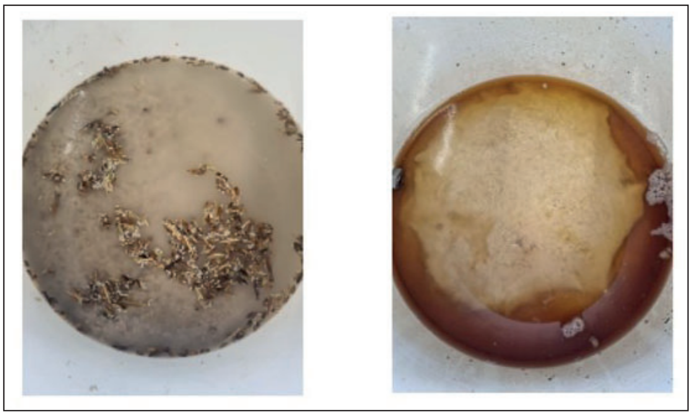

Fig. 1 Turbid Alpine Pfauengerste wort after lautering rest (left) and Alpine Pfauengerste wort after whirlpool rest with a high amount of coagulated protein (right)

ances. Participants were informed that they would taste different lager beers. Samples were served in monadic order in brown beer tasting glasses to avoid the influence of beer colour. 50 mL of beer was served at about 15 °C in randomised order. Unsalted bread (matzo) and water were offered to cleanse their palate.

Liking of beer aroma, flavour, and overall liking were assessed on a 9-point hedonic scale (1 = do not like at all, 5 = neither like nor dislike, 9 = like very much). Willingness to buy was assessed on a 5-point scale (1 = will not buy at all, 5 = would definitely buy). Open-ended response fields were provided for participants to articulate, in their own words, specific aspects they liked or disliked about each beer sample.

After tasting the beer samples blind, participants were informed that the lager beers they had tasted previously were brewed with malt from ancient spring barley varieties, i.e., barley varieties that play only a very minor role in modern agriculture. Participants were informed that these ancient barley varieties were grown and processed locally. Participants were then asked to assess how interesting they find such a concept. They were able to choose between four options: “very interesting”, “relatively interesting”, “of little interest”, and “not interesting at all”. Participants were further asked if they could imagine supporting such a concept by buying this kind of beer. Here, they were able to choose between the following response options: “yes”, “no”, and “not sure”. In a follow-up question, they were asked how much they were willing to pay more for a bottle of 0.5 L, if this beer was brewed with ancient spring barley varieties that were grown, malted, and brewed locally. Here, possible answers were “would not pay more”, “would pay up to 0.50 Euro more”, “would pay between 0.51–1.00 Euro more”, and “would pay more than 1.00 Euro”. Finally, participants had to answer the following question: “I find it particularly relevant that the beers produced in this project contribute to the following…”. Here, multiple answers were possible: “regional produce”, “biodiversity”, “sustainability”, “environmental protection”, or “water protection”.

At the end, socio-demographic data like age (“younger than 20 years”, “20–30 years”, “31–40 years”, “41–50 years”, “51–60 years”, and “older than 60 years”) and gender (“male”, “female”, “nonbinary”) were collected. Also, information about the predominantly consumed beer variety (“lager”, “wheat beer”, “speciality beers”, “Pilsner”, “bock beer”, “others”) and main shopping location for beer (“cash-and-carry drink store”, “food retail store”, “discounter”, “others”) was obtained. Socio-demographic data were gathered through the use of multiple-choice questions. An open-ended response field was provided where participants could state their most preferred beer brand.

Again, for statistical analysis and computer-based data collection FIZZ package 3.7, FIZZ Biosystems, Courtenon, France, was used.

3 Results

3.1 Brewing process and beer analysis

As the quality of the brewing water, i.e., pH-value and mineral concentrations (water hardness), has a large influence on the type of beer brewed [62], the brewing water used was analysed. It had a GH of 2.6 °dH, a CH of 2.35 °dH, NCH of 0.25 °dH, and an RA of 1.7 °dH. Based on these results, the brewing water used can be considered very soft. All mashes were considered iodine neutral at the end of the mashing process, as there was no visual hint for the blue colour of the iodine-starch complex. Mash of Kraffts Riedgerste showed a slightly higher pH-value (5.8) compared to the others, and was therefore reduced to a pH of 5.5 with 80 % lactic acid. During the rest of the brewing process, the wort pH-values of all varieties were comparable. It became apparent that the ancient spring barley variety Alpine Pfauengerste was harder to process than the other ancient barley varieties Mahndorfer Hanna and Kraffts Riedgerste. It caused challenges while transferring the mash into the lauter tun. Also, lautering was more time-consuming. Wort from the ancient spring barley variety Alpine Pfauengerste was very turbid (Fig. 1 left), especially when compared to wort of the modern bred barley variety Avalon. After whirlpool rest, a very high amount of coagulated protein remained (Fig. 1 right). The challenges with the brewing process resulted in a much lower yield (13.5 L) for Alpine Pfauengerste, especially when compared to the modern bred barley variety Avalon industry standard (21.5 L) at the end of the brewing process.

Pale lager beers are characterised by pH-values of 4.3–4.6 [27]. ANOVA results indicate with a confidence level of 99 %, significant differences in terms of pH-value. Lager beer brewed with ancient spring barley variety Alpine Pfauengerste, showed significantly the highest pH-value (4.83) compared to all four other lager beers. The pH-values for beer brewed with Kraffts Riedgerste resulted in a pH-value of 4.61, and Mahndorfer Hanna with 4.65. Lager beers brewed with the modern bred barley variety Avalon resulted in a pH-value of 4.56 (industry standard) and 4.61 (project).

German lager beers are characterised by an alcohol level of 3.5 to 4.5 % w/w [27]. There were also highly significant differences (confidence level of 99.9 %) in alcohol levels. Lager beer brewed with modern bred barley variety Avalon showed significantly higher alcohol levels 5.41 % vol. (project) and 5.37 % vol (industry standard) compared to the ancient spring barley variety Mahndorfer Hanna (4.96 % vol.). Significantly the lowest alcohol levels were found in Alpine Pfauengerste (4.72 % vol.) and Kraffts Riedgerste (4.68 % vol.).

Original gravity (°P) ranged between 11.3 °P and 12.5 °P. ANOVA results showed highly significant differences (confidence level of 99.9 %) between lager beers. Lager beer brewed with the modern bred barley variety Avalon project showed significantly the highest original gravity. No significant differences were found between Alpine Pfauengerste (12.0 °P), Mahndorfer Hanna (11.9 °P), and Avalon industry standard (11.8 °P). Significantly the lowest original gravity (11.3 °P) showed lager beer brewed with the ancient barley variety Kraffts Riedgerste.

Real extract was, with 4.81 %, significantly highest in beer brewed with ancient spring barley variety Alpine Pfauengerste. For Kraffts Riedgerste, real extract value of 4.13 % was measured, and for Mahndorfer Hanna 4.36 %. A slightly higher real extract (4.28 %) was measured in beer brewed with modern bred barley variety Avalon project. Significantly the lowest real extract (3.56 %) was found in lager beer brewed with modern bred spring barley variety Avalon industry standard.

The apparent extract was, with 1.62 %, significantly the lowest for modern bred barley variety Avalon industry standard. While the apparent extract of lager beer brewed with Alpine Pfauengerste was 3.10 % and therefore significantly higher than in all other beers. The measured apparent extract for the other ancient barley varieties, Kraffts Riedgerste and Mahndorfer Hanna, were 2.43 % and 2.56 %. Slightly higher than lager beer brewed with Avalon project, which had an apparent extract of 2.34 %.

Highly significant differences (confidence level of 99.9 %) between the beers were found in apparent attenuation. All three ancient barley varieties had significantly lower apparent attenuation values, compared to modern barley variety Avalon. Ancient barley variety Alpine Pfauengerste had with 74.1 % significantly the lowest apparent attenuation. All other samples showed an apparent attenuation between 78.4 % and 86.3 %. Beer brewed with Avalon industry standard had significantly the highest apparent attenuation of 86.3 %.

Fig. 2 Comparison of lager beer colour from left to right: modern spring barley variety Avalon Industry Standard, Avalon Project, ancient spring barley varieties Alpine Pfauengerste, Kraffts Riedgerste, and Mahndorfer Hanna

The lowest bitterness (20.5 BU) was found in lager beer brewed with the Avalon industry standard, while lager beer brewed with Avalon project showed with 23.8 BU the highest bitterness. The second-highest bitterness (23.1 BU) was found in Mahndorfer Hanna, followed by Alpine Pfauengerste (21.7 BU) and Kraffts Riedgerste (20.8 BU). A bitterness comparable to Avalon industry standard (20.5 BU).

Highly significant differences (confidence level of 99.9 %) in colour were found between the beers tested, where Mahndorfer Hanna and Kraffts Riedgerste showed significantly the lowest EBC colour units and Alpine Pfauengerste the significantly highest EBC colour units. The colour of a pale lager beer is supposed to be between 8–15 EBC colour units [27]. In terms of colour, lager beer brewed with ancient barley variety Alpine Pfauengerste clearly differed from the other beers. With 11.3 EBC, it was significantly the darkest beer. The other ancient barley varieties, Mahndorfer Hanna (5.8 EBC) and Kraffts Riedgerste (5.7 EBC), were clearly below the values given by Eβlinger (2009) [27], i.e., paler than normally expected for pale lager beer. Beer brewed with the industry standard of modern bred barley variety Avalon was characterised by 7.6 EBC colour units, and beer brewed with Avalon project was characterised by

Table 1 Results beer analysis – mean value and standard deviation, Analysis of Variance and Tukey's post-hoc test, at 95 % confidence level

	pH-value	Alcohol (% v/v)	Original gravity (°P)	Real extract (% w/w)	Apparent extract (% w/w)	Apparent attenuation (% w/w)	Bitterness units (BU)	Colour (EBC)	FAN (mg/L)
ANOVA (*p*-value)	0.002 **	< 0.0001 ***	< 0.0001 ***	< 0.0001 ***	< 0.0001 ***	< 0.0001 ***	n.c.	< 0.0001 ***	< 0.0001 ***
Avalon Industry standard	4.56 ± 0.04 b	5.37 ± 0.04 a	11.8 ± 0.00 b	3.56 ± 0.04 c	1.62 ± 0.06 c	86.3 ± 0.5 a	20.5	7.6 ± 0.1 c	180.7 ± 0.03 a
Avalon Project	4.61 ± 0.02 b	5.41 ± 0.00 a	12.5 ± 0.01 a	4.29 ± 0.06 b	2.34 ± 0.06 b	81.3 ± 0.4 b	23.8	8.5 ± 0.2 b	172.2 ± 0.03 a
Alpine Pfauengerste	4.83 ± 0.01 a	4.72 ± 0.01 c	12.0 ± 0.01 b	4.81 ± 0.09 a	3.10 ± 0.09 a	74.1 ± 0.6 d	21.7	11.3 ± 0.0 a	145.7 ± 0.02 b
Kraffts Riedgerste	4.61 ± 0.01 b	4.68 ± 0.06 c	11.3 ± 0.01 c	4.13 ± 0.06 b	2.43 ± 0.04 b	78.4 ± 0.1 c	20.8	5.7 ± 0.2 d	146.0 ± 0.00 b
Mahndorfer Hanna	4.65 ± 0.04 b	4.96 ± 0.04 b	11.9 ± 0.00 b	4.36 ± 0.06 b	2.56 ± 0.08 b	78.5 ± 0.6 c	23.1	5.8 ± 0.2 d	132.1 ± 0.00 b

FAN = free amino nitrogen content, ANOVA = Analysis of Variance, ** significant at 99 % confidence level,, *** significant at 99.9 % confidence level, n.c. = not calculated, Values which do not share the same letter are significantly different

8.5 EBC units, being at the lower end of typical colour values for pale lager beer. Figure 2 shows a comparison of lager beer colour.

Pale lager beers show, on average, a free amino nitrogen (FAN) content of 80–160 mg / L [27]. Highly significant differences (confidence level of 99.9 %) in FAN values were found between the five beers tested. Lager beers brewed with ancient barley varieties showed significantly lower FAN values compared to beer brewed with modern bred barley variety Avalon. Mahndorfer Hanna had with 132.1 mg / L the lowest FAN content, followed by Alpine Pfauengerste (145.7 mg / L) and Kraffts Riedgerste (146.0 mg / L), but all of them were within the ideal range given by Eβlinger (2009) [27]. The highest FAN value (180.7 mg / L) was found in lager beer brewed with the modern bred barley variety Avalon industry standard, followed by Avalon project (172.2 mg / L), and are therefore higher than the values stated by Eβlinger (2009) [27]. A summary of the previously described beer analysis results can be found in table 1.

Table 2 Results difference from control test – Analysis of Variance and Dunnett post-hoc test at 95% confidence level, n = 16 participants, 3 replicates

	Appearance	Aroma	Flavour	Mouthfeel	Aftertaste
Results ANOVA (p-value)	<0.0001 ***	<0.0001 ***	<0.0001 ***	0.0125 *	0.0054 **
Results Dunnett post-hoc test at 95% confidence level					
Alpine Pfauengerste vs. Avalon Industry Standard	sign.	sign.	sign.	sign.	sign.
Mahndorfer Hanna vs. Avalon Industry Standard	sign.	n.s.	sign.	n.s.	n.s.
Kraffts Riedgerste vs. Avalon Industry Standard	sign.	sign.	n.s.	n.s.	n.s.
Avalon Project vs. Avalon Industry Standard	n.s.	sign.	n.s.	n.s.	sign.

Results Dunnett post-hoc test at 95 % confidence level, reference Avalon Industry Standard (n = 15 trained panellists, 3 repetitions), * significant at 95 % confidence level, ** significant at 99 % confidence level, *** significant at 99.9 % confidence level, n.s. = not significant, 6-point scale labelled "no difference to the reference sample" to "very large difference to the reference sample", ANOVA = analysis of variance

3.2 Sensory analysis

3.2.1 Difference from control test

ANOVA results of DFC test indicate highly significant differences (confidence level of 99.9 %) between the samples tested. Dunnett test at confidence level of 95 % for the modality appearance indicates that lager beer from the three ancient barley varieties Mahndorfer Hanna, Kraffts Riedgerste, and Alpine Pfauengerste were perceived as being significantly different from the lager beer brewed with the industry standard of the modern barley variety Avalon. Here, trained panellists reported the darker and more reddish-brown colour of lager beer brewed with Alpine Pfauengerste compared to the reference. In contrast, the lager beers brewed with Kraffts Riedgerste and Mahndorfer Hanna are perceived as being paler than the reference sample. Panellists also reported a more yellowish colour. The appearance of the lager beer brewed with Avalon project was not significantly different from the reference lager beer brewed with Avalon industry standard.

In terms of aroma, ANOVA results showed highly significant differences between samples tested on a significance level of 99.9 %. Dunnett test at a confidence level of 95 % indicated that the lager beers brewed with ancient barley varieties Alpine Pfauengerste and Kraffts Riedgerste were significantly different from the reference (Avalon industry standard), while lager beer brewed with ancient variety Mahndorfer Hanna was not perceived as being different from the reference. Dunnett test results also indicate that the aroma of the lager beer brewed with Avalon project was significantly different from the reference, due to a more intense fruity and banana aroma of Avalon project. Especially the aroma of the lager beer brewed with Alpine Pfauengerste showed the largest difference to the reference. It was described as being more intense in banana and exotic fruit notes. Panellists further described the aroma as being more lactic or buttery, also having more intense vegetative aroma notes, reminiscent of cooked vegetables or cabbage. Lager beer brewed with Kraffts Riedgerste differed from the reference due to more vegetative aroma notes. Here, attributes like cooked vegetables/cabbage and cereal or straw-like aroma notes were mentioned.

For the modality flavour, results of ANOVA showed highly significant differences (confidence level of 99.9 %) between the samples tested. Dunnett test at a confidence level of 95 % for the modality flavour showed that lager beer brewed with ancient barley varieties Mahndorfer Hanna and Alpine Pfauengerste were perceived as being significantly different in flavour to the reference Avalon industry standard. Lager beer brewed with Avalon project and ancient barley variety Kraffts Riedgerste were not perceived as significantly different from the reference. Lager beer brewed with the ancient barley variety Mahndorfer Hanna was described as being more bitter than the reference. Alpine Pfauengerste was described as being more bitter and sweeter and having a more intense cooked vegetable/cabbage flavour together with cereal notes in comparison to the reference.

Taking the modality mouthfeel into consideration, ANOVA showed significant differences (confidence level of 95 %) between samples tested. Results of Dunnett test at a confidence level of 95 % showed that only the ancient barley variety Alpine Pfauengerste was perceived as being significantly different in mouthfeel compared to the reference lager beer from Avalon industry standard. Lager beer brewed with ancient spring barley variety Alpine Pfauengerste was predominantly described as being creamier.

DFC test results for the modality aftertaste showed significant differences (confidence level of 99 %) for the modality aftertaste. Results of Dunnett test at a confidence level of 95 % showed that the ancient barley variety Alpine Pfauengerste was perceived as being significantly different from the reference lager beer brewed with Avalon industry standard. The aftertaste was described as being less lingering compared to the reference. The lager beer brewed with Avalon project was perceived as being significantly different from the reference, because of a more bitter and more lingering aftertaste. No significant difference between the reference and the two ancient barley varieties Mahndorfer Hanna and Kraffts Riedgerste were found. DFC test results were summarised in table 2.

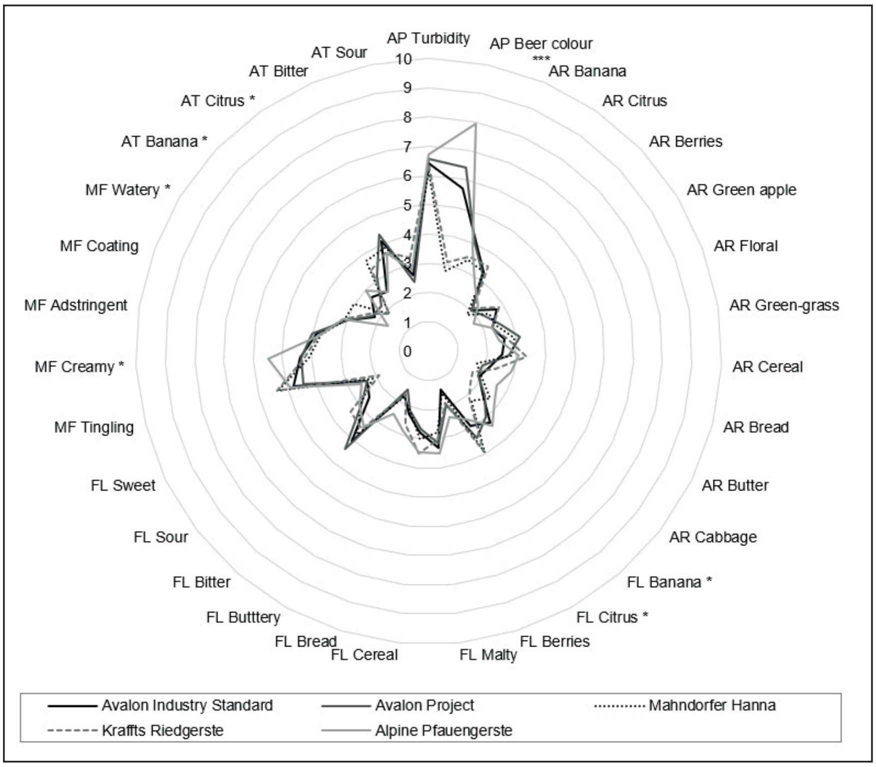

Fig. 3 Analysis of Variance results of descriptive analysis of lager beers – * = significant at 95 % confidence level, * = significant at 99.9 % confidence level, (n = 15 trained panellists, 3 replicates), unstructured line scale from 0–10; AR = aroma, FL = flavour, MF = mouthfeel, AT = aftertaste**

3.2.2 Descriptive analysis

ANOVA results of descriptive analysis data showed highly significant differences (confidence level of 99.9 %) in beer colour. Results of Tuckey test (confidence level of 95 %) showed that lager beer brewed with ancient barley varieties Mahndorfer Hanna and Kraffts Riedgerste were significantly less intense in colour than the two beers from the modern bred barley variety Avalon industry standard and project. Lager beer from ancient barley variety Alpine Pfauengerste was significantly more intense in colour than the two Avalon varieties and the two ancient barley varieties. These results are in line with colour measures, where Kraffts Riedgerste and Mahndorfer Hanna showed the lowest EBC values and Alpine Pfauengerste the highest. ANOVA showed significant differences (confidence level of 95 %) in banana aroma and aftertaste, as well as citrus flavour and aftertaste, but results of Tuckey test indicate no significant differences between the samples tested.

Significant differences (confidence level of 95 %) were found in creamy and watery mouthfeel according to ANOVA results. Tuckey test performed at confidence level of 95 % showed that lager beer brewed with ancient barley Alpine Pfauengerste variety was perceived as significantly more creamy and less watery than the lager beer brewed with the ancient barley variety Mahndorfer Hanna, which was described as being significantly less creamy and therefore more watery in mouthfeel, while lager beer brewed with ancient barley variety Kraffts Riedgerste did not differ from the modern barley variety Avalon. An overview of descriptive analysis results is given in figure 3.

3.2.3 Comparison of hot steep malt sensory evaluation method and check-all-that-apply data

Prior to the brewing trials conducted within this study, the Pilsner malts used were subjected to the preparation of hot steep malt extracts. The preparation of hot steep malt extracts was done following the American Society of Brewing Chemists and Brewers Association: ASBC Sensory Analysis Method 14: Hot Steep Malt Sensory Evaluation [51]. Description of the hot steep malt extracts sensory profile was done by trained panellists, using CATA for the modalities aroma and taste [53]. When comparing the results of CATA data of the hot steep malt extracts sensory profile with descriptive analysis data from the final lager beers, differences among lager beers were smaller than those of hot steep malt extracts.

While hot steep extracts from Kraffts Riedgerste and Alpine Pfauengerste showed vegetative notes like grass, tea or cucumber (see Fig. S1 and Fig. S2) [53], neither Kraffts Riedgerste nor Alpine Pfauengerste showed a significantly more intense vegetative aroma in the final beer. The aroma of hot steep extract from Alpine Pfauengerste was often described by the attribute "other", which also included atypical notes. The taste of hot steep extract from Alpine Pfauengerste was further characterised by sour and bitter notes. Lager beer brewed with the ancient barley variety Alpine Pfauengerste was also described as having a more intense bitter flavour when compared to the reference Avalon industry standard within the DFC test, but the sour taste was not reported in the final lager beer brewed with Alpine Pfauengerste. Panellists also described lager beer brewed with ancient spring barley variety Alpine Pfauengerste as having more intense cooked cabbage notes compared to the reference. Hot steep malt extracts from Avalon industry standard were characterised by a fruity and honey aroma and Avalon project by notes of berries, but lager beers brewed with Avalon project and industry standard were, according to descriptive analysis data, not significantly higher in these aroma notes compared to lager beer brewed with the three ancient barley varieties.

3.2.4 Consumer acceptance test

Lager beer samples were assessed by 100 participants who consumed beer at least once per month or more often. The majority (39 %) consumed beer several times per week. An additional 30 % stated that they consume beer at least once per week, and 24 % several times per month. Only five percent

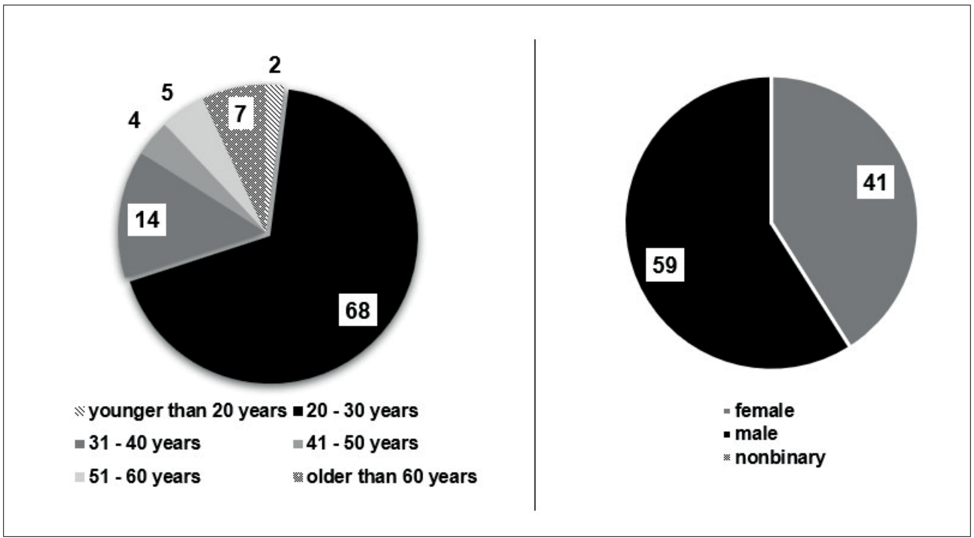

Fig. 4 Age structure (left) and gender structure (right) of participants in percentage (n = 100)

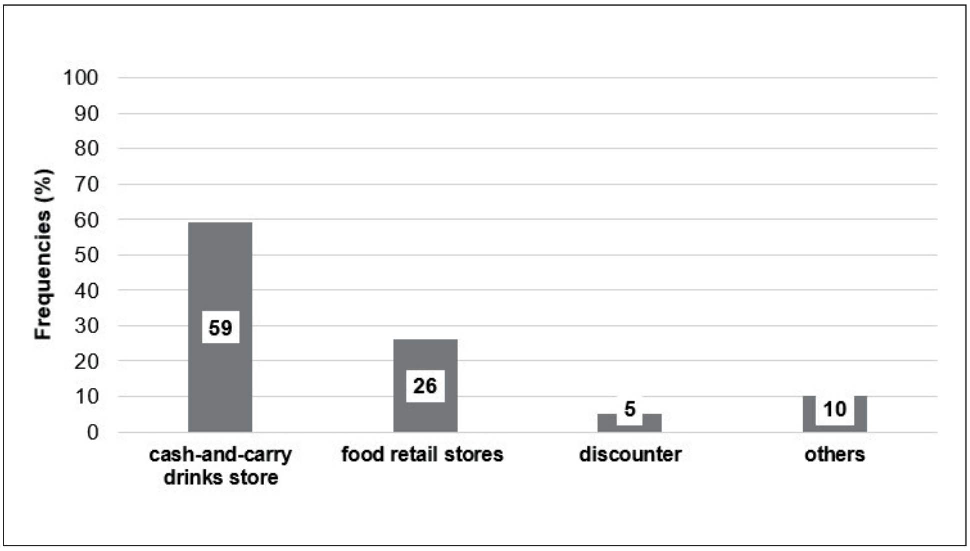

Fig. 5 Participants predominant shopping location for beer (n = 100)

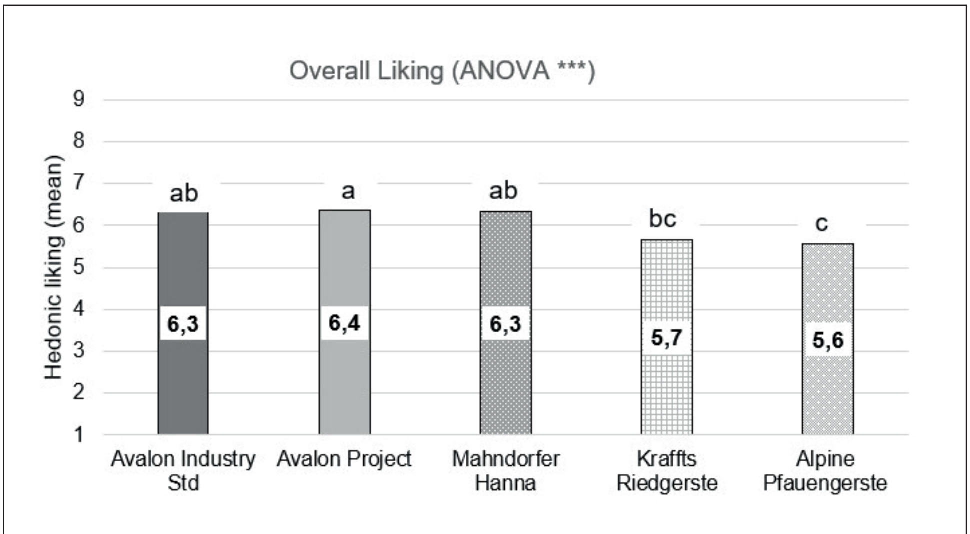

Fig. 6 Overall liking lager beer; Analysis of Variance (ANOVA) results: *** significant at 99.9 % confidence level, followed by Tukey post-hoc test at 95 % confidence level, (n = 100), 9-point hedonic scale (1 = do not like at all, 5 = neither like nor dislike, 9 = like very much), values which do not share the same letter, are significantly different

stated that they consume beer at least once per month. Only two percent less regularly.

Regarding the socio-demographic structure, 59 % of participants were male and 41 % female; no one stated their gender as “nonbinary”. In terms of age structure, the majority (68 %) of participants were between 20–30 years of age, 14 % between 31–40 years, 7 % were older than 60 years, 5 % were between 51–60 years, 4 % were between 41–50 years; only 2 % were younger than 20 years (see Fig. 4).

As shown in figure 5, the majority of participants (59 %) buy their beer predominantly at retail cash-and-carry drink stores. For 26 % of the participants, food retail stores are their main shopping location for beer, while discounters play only a minor role. Only 5 % stated that they buy their beer predominantly there. When the attribute “others” was selected, the majority, i.e., six out of ten, wrote that they buy their beer in a so-called kiosk – a common feature in German cities. The term kiosk describes a small, often independently operated shop similar to a British corner shop, offering a selection of alcoholic and non-alcoholic beverages, snacks, tobacco, and newspapers.

When asked about their most preferred type of beer, the majority of participants stated that they prefer to drink Pilsner or other lager-type beers, followed by wheat beer and specialty beer. Participants were also asked (in open text fields) which beer brands they prefer: there was no clear favourite, and not only larger brands were mentioned. 14 times Bitburger was named; the second most important beer brand in Germany in 2025 in terms of brand awareness [63], while some (10x) preferred Veltins, ranked 8th among the largest beer brands in Germany [63]. 9 times it was stated that they did not prefer a specific brand, 8 times Chiemseer was mentioned, a brand that belongs to Paulaner Brauerei Gruppe GmbH & Co. KGaA. Further, 8 x Paulaner was named, which was ranked 5th among the largest beer brands in Germany in terms of brand awareness [63], followed by 7 x Rothaus (Badische Staatsbrauerei Rothaus AG), positioned in 20th place among the largest beer brands in Germany in terms of brand awareness [63]. Kormbacher, in terms of brand awareness, the largest beer brand in Germany in 2025 [63], as well as Bayreuther (Bayreuther Bierbrauerei AG) and Flensburger (Flensburger Brauerei Emil Petersen GmbH & Co. KG) were named 6 times. Störtebecker (Störtebeker Braumanufaktur GmbH) was mentioned 5 times by participants. Additional beer brands of small and medium sized breweries were reported only in isolated cases.

When participants were invited to rate their liking of each beer tasted, ANOVA results showed highly significant differences (confidence level of 99.9 %) in "overall liking". Results of the post-hoc Tukey test, at confidence level of 95 %, showed that overall lager beer brewed with the ancient spring barley variety Mahndorfer Hanna was liked as much as lager beer from the modern barley variety Avalon (industry standard and project). Lager beer from ancient spring barley variety Kraffts Riedgerste was overall liked as much as Avalon industry standard, Mahndorfer Hanna, and Alpine Pfauengerste. Lager beer brewed with malt from the ancient spring barley variety Alpine Pfauengerste was overall significantly less preferred than lager beer from ancient barley variety Mahndorfer Hanna and the two beers brewed with the modern bred barley variety Avalon (see Fig. 6).

When participants were asked how much they liked the aroma and flavour of the respective beer, results indicate significant differences (confidence level of 95 %) in liking of the aroma and flavour of the lager beers. While results of the Tukey Test at a confidence level of 95 % showed no significant differences in liking of the aroma of the beers tasted (Fig. 7), it can be stated that the aroma of the ancient spring barley varieties Mahndorfer Hanna, Kraffts Riedgerste, and Alpine Pfauengerste were liked as much as the aroma of the beer from modern barley variety Avalon project and industry standard.

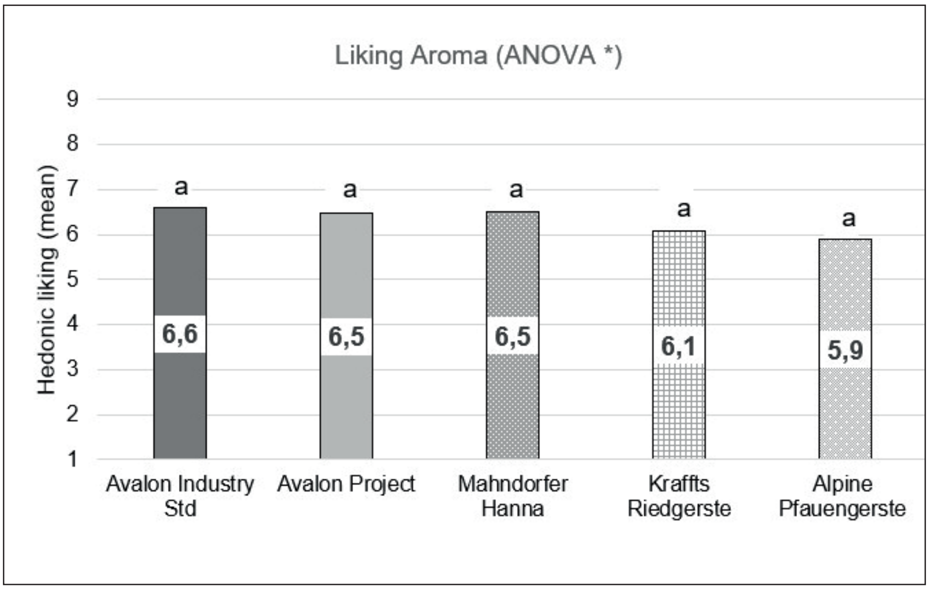

Fig. 7 **Liking lager beer aroma, Analysis of Variance (ANOVA) results: * significant at 95 % confidence level, followed by Tukey post-hoc test at 95 % confidence level, (n = 100), 9-point hedonic scale (1 = do not like at all, 5 = neither like nor dislike, 9 = like very much), values which do not share the same letter, are significantly different**

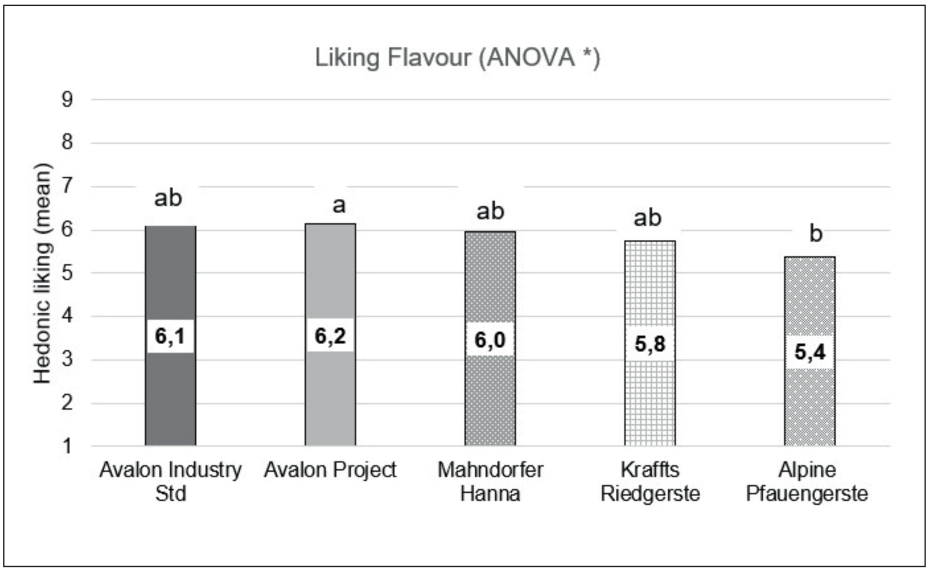

Fig. 8 **Liking lager beer flavour; Analysis of Variance (ANOVA) results: * significant at 95 % confidence level; followed by Tukey post-hoc test at 95 % confidence level, (n = 100), 9-point hedonic scale (1 = do not like at all, 5 = neither like nor dislike, 9 = like very much), values which do not share the same letter, are significantly different**

Results of Tukey Test at 95 % confidence level showed significant differences in flavour liking. The flavour of the two ancient spring barley varieties, Mahndorfer Hanna and Kraffts Riedgerste, was liked as much as the flavour of the modern barley variety Avalon (industry standard and project). Only the flavour of the ancient spring barley variety Alpine Pfauengerste was significantly less preferred than the taste of the lager beer with the modern spring barley variety Avalon (project) (see Fig. 8).

Participants liked lager beer from the ancient barley variety Mahndorfer Hanna due to its pleasant aroma (19 x), its fruit aroma (6 x), and taste (7 x). This beer was described as well-balanced (8 x) and pleasant to drink (11 x).

Eleven participants liked the aroma of lager beer from Kraffts Riedgerste. An additional eight participants specified that they liked its fruity aroma. This beer was further liked due to its well-balanced taste (6 x), pleasant bitterness (9 x), and fruitiness (4 x). Lager beer brewed with ancient barley variety Kraffts Riedgerste was also liked due to its CO_2 content (10 x), which made it pleasant to drink (4 x).

Beer brewed with the ancient barley variety Alpine Pfauengerste was liked by 13 participants due to its pleasant aroma, and a further eight participants liked its fruity aroma and taste (5 x). They liked the beer's pleasant, not too intense bitterness (9 x) and its

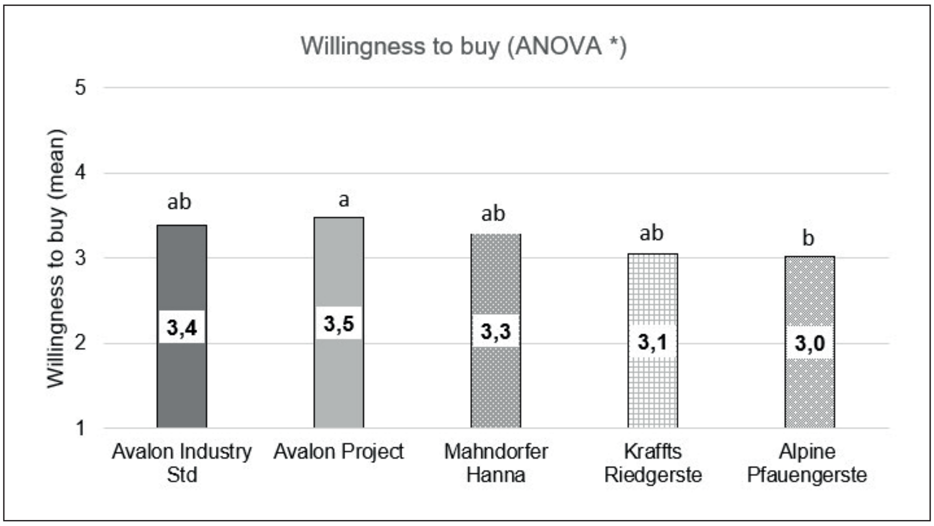

Fig. 9 **Willingness to buy lager beer, Analysis of Variance (ANOVA) results: * significant at 95 % confidence level, followed by Tukey post-hoc test at 95 % confidence level, (n = 100), 5-point scale (1 = will not buy at all, 5 = would definitely buy), values which do not share the same letter, are significantly different**

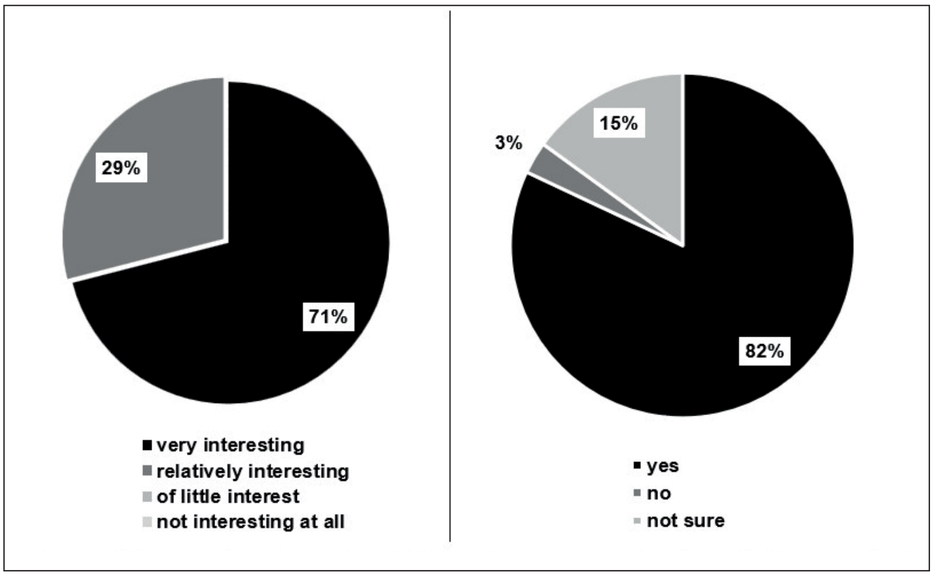

Fig. 10 **Participants' interest in a concept of beer brewed with ancient barley varieties, which are grown and processed locally (left), Participants' willingness to support a concept of beer brewed with ancient barley varieties if they are grown and processed locally (right), (n = 100)**

maltiness (7 x). Participants also liked the beer's pleasant mouthfeel (9 x), CO_2 content (9 x), and drinkability (5 x). Participants perceived this beer as well-balanced (5 x).

Participants stated that they predominantly liked the aroma (6 x), fruity aroma (11 x), and fruity taste (5 x) of the lager beer brewed with Avalon industry standard. They also perceived the aroma as pleasant (8 x) and its taste (8 x) as well. Participants also liked the beer's pleasant bitterness in taste (10 x), maltiness (6 x), and mouthfeel (6 x); they further considered it pleasant to drink (5 x). For beer brewed with Avalon project, participants perceived its aroma as pleasant (15 x), and they liked its fruity (7 x) and malty (5 x) notes in aroma. They like the beer's pleasant bitterness (15 x) and acidity (5 x), its taste in general (8 x), its fruitiness (5 x), and mouthfeel (7 x). The beer was considered as pleasant to drink (6 x).

When considering the dislikes of modern spring barley varieties, participants perceived the lager beer brewed with the modern barley variety Avalon (project) as too bitter in taste (18 x) and aftertaste (5 x). Participants did not like the aftertaste in general (5 x), as well as the beer's intense sourness (5 x) and low flavour intensity (5 x). Regarding the dislikes of the lager beers brewed with Avalon (industry standard), they reported a low aroma intensity (12 x) and low taste intensity (11 x). Further, the taste was perceived as too bitter (10 x). The same holds for the aftertaste (4 x).

Regarding the beers brewed with ancient barley variety Mahndorfer Hanna, participants stated that they did not like the too intense bitter taste (10 x) and aftertaste (4 x). They perceived its aroma as not intense enough (14 x). Atypical beer flavour (4 x), vegetative notes (5 x), and a not intense enough flavour (5 x) were stated, too.

For Kraffts Riedgerste, aroma (13 x) and taste (13 x) were perceived as not being intense enough. The beer was further perceived as too bitter in taste (10 x) and aftertaste (4 x), as well as being too watery in mouthfeel (7 x).

When considering the "dislikes" for lager beer brewed with the ancient spring barley variety Alpine Pfauengerste, participants were bothered by the beer's intense bitterness in taste (14 x) and aftertaste (5 x). Further participants perceived the taste as not intense enough (8 x), they did not like its acidity (8 x) and notes of corn/asparagus (7 x). Further the aroma was perceived as musty (4 x), vegetative (5 x) and lactic (4 x), which might indicate why this lager beer was significantly liked less compared to the other varieties.

After tasting each beer, participants rated their willingness to buy on a 5-point scale. Results of the ANOVA showed significant differences (95 % confidence level). Participants indicated that they were significantly less willing to buy lager beer brewed with ancient barley variety Alpine Pfauengerste. While there were no significant differences between beer brewed with the modern barley variety Avalon and the ancient barley varieties Mahndorfer Hanna and Kraffts Riedgerste. Overall, the willingness to buy ratings were across all beers tasted not extremely high (Fig. 9).

After tasting the samples blind, participants were informed about the origin of the samples tasted. They were told that the lager beers were brewed with ancient spring barley varieties, which play only a very minor role in today's agriculture. Participants were also notified that these varieties were cultivated and brewed locally, with the aim of selling the beers locally. After being provided with these details, participants were asked how interesting such a concept would be to them. The majority (71 %) found the concept very interesting, while 29 % found it relatively interesting. In a follow-up question, participants were asked if they would support such a concept. And a great majority (82 % of all participants) would support such a concept. While 15 % were unsure, only 3 % would not support such a concept (Fig. 10).

As production costs of beer brewed with ancient spring barley varieties are much higher compared to modern barley varieties, participants were asked about their willingness to pay. They were asked how much more they are willing to pay for a 0.5 L bottle of beer from ancient barley varieties in a food retail store. 40 % would pay up to 0.50 Euro more per bottle, while 40 % would pay between 0.51 Euro to 1.00 Euro more per 0.5 L bottle of beer brewed with ancient barley varieties. 11 % are willing to pay more than 1.00 Euro per bottle, while 9 % are not willing to pay more for such a type of beer (see Fig. 11).

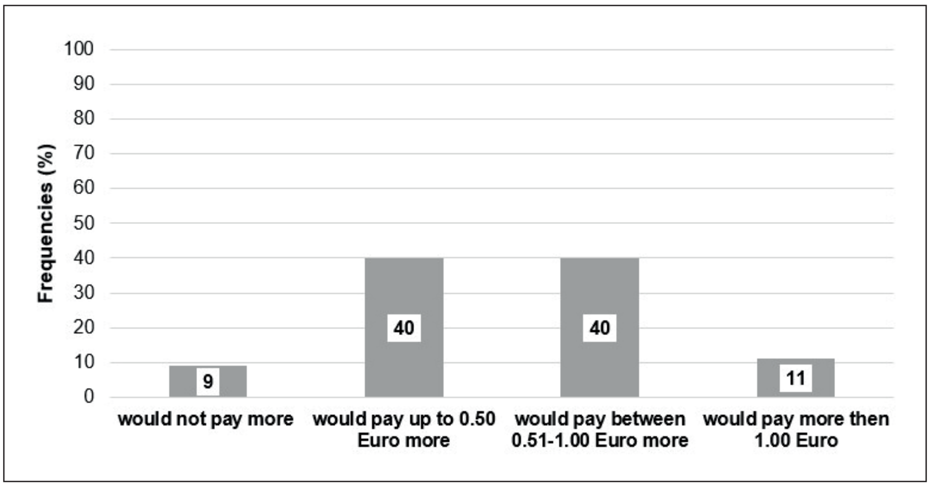

Fig. 11 **Frequency of participants' willingness to pay more for a 0.5 L bottle of lager beer (in a food retail store) from ancient barley varieties, if it is grown, brewed, and produced locally (n = 100)**

The questionnaire concluded with the question: "I find it particularly relevant that the beers produced in this project contribute to the following...: "regional produce", "biodiversity", "sustainability", "environmental protection", and "water protection". Participants' selection looked as follows: 80 % stated that sustainability is a relevant aspect, followed by regional produce (77 %). Less important, but still relevant, are protection of the environment (58 %) and biodiversity (54 %), while water protection was mentioned by 40 % of the consumers (see Fig. 12).

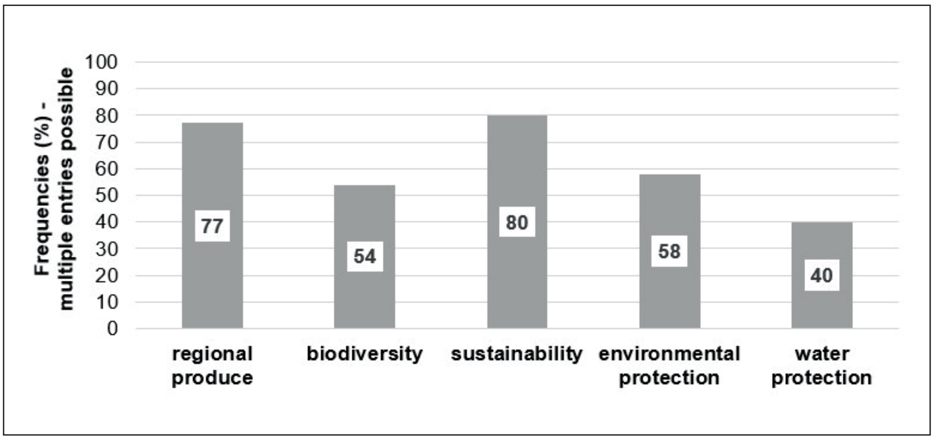

Fig. 12 **Frequencies of participants' responses towards "I find it particularly relevant that the beers produced in this project contribute to the following..."; multiple entries possible (n = 100)**

4 Discussion

4.1 Brewing process and beer analysis

The quality of brewing water used within this study was described as "very soft" and therefore suitable for brewing Pilsner beer. Kunze (2023) considered brewing water with a GH between 0.1–4 °dH as "very soft" and recommends that for the production of Pilsner beer, the residual alkalinity should not exceed 2 °dH, too [64]. Following the international hardness scale of water, the brewing water used within this study falls under the degree of hardness level 1 (< 7 °dH) and is therefore considered "soft". For the production of beer with a pale colour, the residual alkalinity should not be higher than 5 °dH. Higher residual alkalinity might lead to a higher pH-value and to a more intense wort and beer colour [54, 64].

Brewing with ancient spring barley varieties might be less streamlined than brewing with modern bred spring barley varieties, especially with Alpine Pfauengerste. Brewing with ancient spring barley variety Alpine Pfauengerste resulted in challenges during solid-liquid separation and a very high amount of coagulated protein after whirlpool rest. This might be explained by the high raw protein content of this ancient barley variety [53]. Also, other authors reported higher raw protein content in ancient barley varieties [65, 66]. Despite the negative impact of raw high protein content in barley on brewhouse performance, Wannenmacher et al. (2019) stated that beer brewed with malt originating from barley with a higher raw protein content, resulted in lower oxidative aging as well as a significantly higher antioxidant activity [67].

At the end of the mashing process, all mashes were considered as iodine neutral, which refers to sufficient starch degradation. An insufficient conversion might lead to low extract yields, turbidity caused by starch haze, problems during filtration and reduced final fermentation degrees [64]. Alpine Pfauengerste was predominantly described as a showing a very turbid wort after solid-liquid separation. Many factors may have an influence on lauter turbidity, for instance, raw material, lauter equipment, the height of the filter bed, raking, sparging, as well as the speed of lautering [68]. The later was very slow with this variety. A high level of trub in wort will stimulate yeast activity, and due to that, rate of fermentation.

Higher levels of trub have an influence on flavour formation, where fewer esters but more fusel alcohol were produced, which resulted in lower acceptance by consumers [69].

Mash of Kraffts Riedgerste showed slightly higher pH-value (5.8) compared to the mash of other barley varieties, and was therefore reduced to a pH of 5.5 with 80 % lactic acid, as according to Kunze (2023), the ideal pH-value for mash is between 5.5 and 5.6 in terms of attenuation limit, protein breakdown, viscosity, lautering rate, and restriction of colour increase [64]. A higher pH-value in mash might lead, for instance, to a higher extraction of colouring materials and, according to Comrie (1967) also an undesired extraction of polyphenols leading to unpleasant, bitter taste as well as the risk of turbidity [70]. As pH-value in mash was adjusted with 80 % lactic acid rather than with sour wort, the beers within this study are not in line with the German Purity Law.

Original gravity of lager beers brewed within this study, with the exception of Kraffts Riedgerste, are comparable to original gravity values reported by Olšovská et al. (2018), where beers brewed with the Czech ancient barley variety Proskovcuv hanack, resulted in an original gravity of 12.13 % and 12.45 % [65]. The high extract content of the ancient barley variety Alpine Pfauengerste might not be explained by its high protein content of 17.1 % [53]. Normally each percentage of higher protein content results in the same percentage of less extract [64]. The high protein content might be the reason why this variety was harder to process, as a high protein content might also lead to difficulties during filtration and might cause turbidity [64, 71]. However, higher protein contents might increase the performance of beer foam [72]. Mikyška et al. (2022) showed that beer brewed with ancient spring barley varieties showed lower extract yields than beer brewed with modern spring barley varieties [66]. Olšovská et al. (2018) also conducted brewing trials with an ancient Czech barley variety, however, using floor malting and decoction method for mashing, which resulted in beers with an apparent attenuation of only 67 % and 69 % [65]. All lager beers brewed with the three ancient spring barley varieties showed significantly lower apparent attenuation than beer brewed with modern spring barley variety Avalon, but are higher than values reported by Olšovská et al. (2018). The bottom-fermented beers, from the ancient Czech barley variety, also showed relatively low alcohol content of 3.40 and 3.57 % by weight. Beers were further characterised by higher levels of dextrins and β-glucans, arabinoxylans, and polyphenols. Sensory evaluation of the beers was similar to current conventional lager-type beers, showing a sensory profile of typical lager beer with aroma of yeast and slight hop and perfume notes in aroma and taste. Despite the high polyphenol content, the beers from the ancient Czech barley variety were not perceived as being much more astringent [65].

When taking the description of Eβlinger (2009) [27] for lager beer into consideration in terms of bitterness, all beers within this study were above the average of 20 EBC (BU) for pale lager beers. While Schmelzle et al. (2011) reported a broader range in German Pilsner beers of 21 to 42 BU [73]. The beers brewed within this study are at the lower end of this range. Schmelzle et al. (2011) stated that consumers did not like Pilsner beer with an intense bitterness of > 34 BU in blind tasting situations. In a branded taste environment, consumers accepted a higher bitterness [73].

Olšovská et al. (2018) reported FAN values of 135 mg / L and 144 mg / L in their beers [65]; however, taking into consideration that these beers were produced with decoction method, while within this study infusion mashing was used. The FAN content of the final beer has an influence on beer aging. High FAN levels might result in Strecker aldehydes, which can contribute to the staling flavour in beer. For higher flavour stability, a lower residual FAN content in the final beer should be targeted [74]. Strecker aldehydes are, for example, benzaldehyde (almond, cherry-stone), methional (cooked potatoes, wort-like), 2-methylpropanal (grainy, varnish, fruity), 2-methybutanal (almond, apple-like, malt-like), 3-methylbutanal (malt-like, chocolate, cherry, almond), and phenylacetaldehyde (hyacinth, floral, rose) [75, 76].

The darker colour of beer brewed with the ancient barley variety Alpine Pfauengerste might be due to a higher oxygen intake because of difficulties when transferring the mash into the lauter tun. As stated by O'Rourke (2002), an increased oxidation within the brewing process will lead to a more intense colour of the wort and final beer through oxidation of polyphenols [77]. Another explanation for the intense colour (11.3 EBC) of lager beer brewed with Alpine Pfauengerste might be protein overmodification be caused by the standardised malting and mashing process used within this study.

Due to the challenges encountered in the brewing process, especially with the ancient spring barley variety Alpine Pfauengerste (solid-liquid separation, large amount of aggregated protein after wort boiling, turbid wort), it is recommended to properly adjust the malting, as well as the brewing process parameters to each ancient barley variety. As within this study a malting process with standardised process parameters was used, it is recommended for further studies to adjust the malting process, to each variety's requirements. Adjusting malting parameters, for instance steeping time and temperature, will result in higher malt quality. Which will ensure better brewhouse performance and an improved sensory profile of the final beer. In terms of adjustments regarding the brewing process, at least a traditional standard three-step infusion mashing should be applied when brewing with ancient barley varieties. Three-step infusion mashing includes a β-glucanase and protein rest at around 45 °C, a β-amylase rest at approximately 62 °C, as well as an α-amylase rest at 70 °C [68]. Nowadays, often only a two-step infusion mashing is applied, because modern bred barley varieties and highly-modified malt allow mashing in directly at temperatures < 60 °C, because proteins are already broken down [14]. Mashing for light beers needs only adjustment for amylolysis [78]. The so-called decoction mashing method, which is suitable for under-modified or enzymatically weak malt, and mainly used to produce lager beer [79] might also be an option when brewing with ancient spring barley varieties. An advantage of the decoction method is the support of an enzymatic breakdown by physical-thermal decomposition of starch with the help of the boiling parts of the mash [80]. Of course, the three-step infusion mashing, as well as the decoction mashing, are less efficient in terms of time, labour, and energy consumption [79]. Based on the results of this study, the use of ancient spring barley varieties will not be suitable for large-scale industrial breweries, but rather for craft beer breweries to extend their product range with interesting new flavour characteristics [65, 66]. However, further research is needed in terms of tailoring the malting and brewing processes to each variety, which will improve the quality and sensory profile of the final beer. Adjusted process parameters might also improve the rating for Alpine Pfauengerste. The three ancient barley varieties studied here might be interesting for craft brewers, as their consumers evaluate the use of ancient barley varieties, especially when grown locally, as beneficial [81]. It might also be recommended to use ancient spring barley varieties for the production of unfiltered beer, as filtration rates during congress mashing procedure were relatively low [53]. Also, Mikyška et al. (2022) found lower filtration rates in beer brewed with ancient barley varieties [66]. Craft beer breweries also tend to avoid filtration and pasteurisation [11, 12], so the use of ancient barley varieties might be an option for them, despite some craft brewers tend to filter their lager beers, too. Mikyška, (2022) assessed the ancient Czech varieties studied in his project as "well processable in the pilot brewery" when using decoction mashing [66].

4.2 Sensory analysis

From a sensory point of view, there were differences between the beers. DFC test results indicated differences in appearance. The more intense colour of lager beer brewed with Alpine Pfauengerste, might be caused by an increased oxidation within the brewing process [77] due to complications when transferring the mash to the lauter tun, but might also be caused by protein overmodification. The sensory description is in line with the analytical analysis. Lager beer brewed with the ancient barley variety Alpine Pfauengerste had the highest EBC colour units of all beers tested.

Panellists description of a paler colour of lager beer brewed with ancient barley varieties Kraffts Riedgerste and Mahndorfer Hanna is in line with analytical results, as both varieties showed lower EBC colour units compared to the Avalon industry standard reference.

DFC test results also indicate differences in aroma. A banana-like aroma, which was described for Avalon project, and which was more pronounced in it, compared to the reference Avalon industry standard, might be due to iso-amyl acetate. With levels typically ranging from 0.5 to 5 mg / L. The taste threshold lays at 1.2 mg / L. Another aroma component referring to a banana-like aroma is iso-butyl acetate with a taste threshold of 1.6 mg / L [61]. As the DFC test results of aroma and flavour of lager beer brewed with ancient barley variety Alpine Pfauengerste, was perceived as being very different to the reference Avalon industry standard, due to more intense lactic or buttery aroma, this might be due to high concentrations of the vicinal diketones diacetyl and 2,3-pentandione, which are often responsible for rancid, cheese-like, or butter-like off-notes in beer. The taste threshold for diacetyl ranges from 0.08 to 0.2 mg / L, while the taste threshold of 2,3-pentandione is between 0.5 and 0.6 mg / L, depending on the type of beer [27]. Notes of cooked vegetables and cabbage reported in aroma and flavour of lager beer brewed with ancient barley variety Alpine Pfauengerste, might be due to high levels of DMS. Some DMS precursors like S-methyl methionine (SMM) are already formed during malting, some dimethyl sulfoxide (DMSO) are influenced by wort boiling and fermentation [82, 83]. DMS is predominantly formed during wort boiling and hot trub separation by thermal degradation of SMM. DMS might also be formed from DMSO. DMSO itself has no influence on beer flavour, but functions as a secondary DMS precursor during fermentation as it is broken down by yeast. While SMM is heat sensitive, DMSO is heat stable and might survive the wort boiling process [82, 84]. While moderate levels of DMS are favourable in lager beers, higher concentrations might be considered as unpleasant [85].

When taking the DFC test results of all modalities (aroma, flavour, mouthfeel and aftertaste) together, it can be concluded that the sensory profile of lager beer brewed with ancient barley variety Alpine Pfauengerste was perceived as being most different compared to the reference brewed with Avalon industry standard, while beers brewed with the ancient barley varieties Kraffts Riedgerste and Mahndorfer Hanna only showed slight differences in their aroma profile compared to the reference.

It can also be concluded that using DFC test to determine sensory differences in lager beer is a suitable tool, especially when a reference is available for direct comparison. Having open-ended response fields where panellists can describe the sensory differences they perceive is also helpful for better understanding how the respective sample differs from the reference. However, the application of DFC test requires extensive training in the use of the scale. It must also be taken into consideration that the sensory profile of pale lager beer is unstable, as it changes during its shelf life [86]. Indicators of beer aging, from a sensory point of view, are furfural, hexanal, 2-methylpropanal, 2-methylbutanal, and 3-methylbutanal [87].

The significantly more creamy mouthfeel of lager beer brewed with ancient barley variety Alpine Pfauengerste might be due to its higher protein content. Beer with a high protein content results in a full-bodied mouthfeel [88, 89]. As shown by Häge et al. (2025), the ancient barley variety Alpine Pfauengerste had a much higher protein content compared to the other varieties included in this study, which might be the reason for the less watery and the more creamy mouthfeel [53]. The less creamy mouthfeel of Mahndorfer Hanna and Kraffts Riedgerste might be due to their lower alcohol content, as alcohol is an important contributor to a full-bodied mouthfeel [90, 91], too.

Results of this study are in line with findings from Mikyška et al. (2022), who stated that beer brewed with ancient spring barley varieties resulted in comparable sensory quality, assessed by the beer's overall impression, to beer brewed with modern Czech spring barley varieties. However, in terms of cluster analysis, the ancient barley varieties were differentiated from the modern bred spring barley varieties [66].

Results of this study have to be verified with further brewing trials using Pilsner malt from ancient barley varieties, originating from different growing locations as well as vintages, despite the fact that Morrissy et al. (2022) did not find an influence of barley genotype or growing location on beer flavour [92]. By contrast, Herb et al. (2017) showed in micro-malting and nano-brewing trials a significant influence of barley genotype on sensory descriptors, for instance, cereal, floral, fruity, grassy, malty, toasted, toffee, and sweet notes, as well as beer colour [93].

But other factors, like fermentation, might influence the flavour of the final beer, too. For instance, Rosales et al. (2021) found a more intense fermentation taste in craft beers in comparison to commercial beer, which might be caused by a maturation (or second fermentation) in the bottle, which is common in craft beer brewing; also, the avoidance of a pasteurisation process might explain this characteristic attribute given by yeast [94]. Neither in the descriptive analysis nor in the DFC test intense yeast notes were reported by panellists, despite the fact that lager beers of this study underwent a second fermentation in the bottle and were not pasteurised, too. A second fermentation in the bottle is on the one hand not common for lager beer, and on the other hand might alter the flavour of the final beer, too. The flavour differences detected within this study might not be solely attributed to barley variety. Due to that, in further studies a second fermentation should be avoided to see more in detail the influence of barley genotype on beer flavour. It is also recommended to conduct further studies with unfiltered beer to avoid masking barley genotype induced flavour differences by yeast attributes. Gonzalez Viejo et al. (2019) concluded that bottom-fermented beers, in this case, mainly lager beers, are less complex in aroma in comparison to top and spontaneous fermented beers. They resonate this with the strain of yeast used, lower fermentation temperatures, as well as less hop/hop extract used. Mainly fruity aroma compounds, reminiscent of apple, pineapple, and grape, were found in bottom-fermented beers. Only two compounds, which are associated with rose and honey, were found [95]. A grain-like or straw-like aroma might be due to an inefficient or too short kettle boil [61]. Within this study, no significant differences in the aroma and flavour attribute "cereal" were found.

4.3 Comparison of results from hot steep malt sensory evaluation method and check-all-that-apply data with results from descriptive analysis of final beers

When comparing the sensory profiles of hot steep malt extract with the sensory profiles of the final beer, it can be stated that hot steep malt extracts were more distinct than the sensory profiles of the final lager beers. The lager differences found in hot steep malt extracts compared to the sensory differences found in the final lager beers are in line with Windes et al. (2021), who stated that they found some attributes like "sweet bread" in hot steep malt extracts as well as in the final beer, but the sensory profile of hot steep malt extracts were more distinct than the sensory profile of the final beers [50]. This might be due to the fact that within this study an unfiltered beer was used, which might mask flavour differences caused by barley variety, as fermentation by-products, as well as the addition of hop during the wort boiling process, will influence a beer's sensory profile to a large extent. Differences might be masked by volatile aromatic compounds originating from hops. The addition of hops can result in floral/citrus-like notes (linalool, geraniol), spicy or clove-like notes (β-caryophyllene, β-eudesmol), herbal, wooden, or resinous-like notes (myrcene), but also tropical fruit notes [96, 97]. The aroma composition also depends on the variety used and the time of hops addition [97]. However, differentiating volatile aroma compounds originating from hops and those formed during fermentation is often complex [96]. Fermentation by-products, with an important influence on the sensory profile of the final beer, are, for instance, carbonyl compounds, higher alcohols, esters, organic acids, as well as sulphur-containing compounds [98, 99]. Kumar et al. (2023) also showed that the yeast strain used has an influence on the modulation of the hop flavour in beer, too [100]. For further studies it is recommended to work with unfiltered beer and beer that has not undergone a secondary fermentation in the bottle. This would facilitate the identification of flavour differences attributable to the barley variety by minimising confounding variables and avoiding flavour alteration.

Following the conclusion of Windes et al. (2021) [50], and based on the results of this study, it must be stated that more evidence is needed to determine if the sensory profile of hot steep malt extracts can predict the flavour of the final beer. This would be a great advantage for maltsters and brewers, but so far, the application of HSME in combination with CATA is suitable for quality control of malt and detection of off-notes in base malt [53], but so far, it cannot be recommended for the prediction of final beer's aroma and flavour.

4.4 Consumer acceptance test

To conduct consumer acceptance tests is reasonable, as a consumer's response towards a product is important. In the end, the success of a product depends on its ability to meet consumers expectations. The consumer acceptance test within this study was conducted with 100 consumers, and as craft beer consumers are normally younger than 40 years [101], it is also justifiable to have more younger consumers integrated in this study. Carvalho et al. (2018) showed in their study on craft beer consumers that 81.7 % were aged between 19 and 39 years [6]. Albert (2025) stated that the age group of 26 - 44 years rated craft beer more favourable than other age groups [102].

Rivaroli et al. (2019), who studied gender-specific behavioural patterns in craft beer consumption in Italy and Germany, concluded that there are still, to some extent, gender differences in Germany. There is "still masculine value system regarding beer consumption" [103]. So, it is reasonable to have slightly more male consumers integrated in this study.

Within this study the shopping behaviour of participants was studied, too. Where it became obvious that majority bought their beer in so called cash-and-carry drink stores, followed by food retail stores. Meyerding et al. (2019) reported in their study, that the majority of German participants (67.6 %) bought their beer in supermarkets, which might be similar to retail food stores. The authors reported beverage markets (57.9 %) as another relevant shopping location for beer. Another third (33.1 %) stated they buy their beer in discounters [104], whereas within this study, only 5 % named discounters as their main shopping location for beer. Based on the results given, it is recommended for beer brewers working with ancient spring barley varieties, to try to market their beer via cash-and-carry drinks stores or food retail stores. According to Meyerding et al. (2019), beer is mainly consumed at home (82.0 %), while restaurants (38.6 %) and pubs (33.9 %) play a minor role [104]. However, when focusing on craft beer consumers only, results might be different: Aquiliani (2015) concluded that craft beer is mainly consumed in pubs, brewpubs, or restaurants and with family members [24]. Therefore, it might be feasible to sell beer brewed with ancient spring barley varieties as draft beer, where production costs are slightly lower, as no bottling and packaging is needed. This might compensate for some of the higher production costs, caused by brewing with ancient spring barley varieties.

Results of this study showed that the majority of participants preferred Pilsner beer and other type of lager beer followed by wheat beer. In terms of consumer preferences for beer, the results of this study align with others, for instance, Meyerding et al. (2019), who studied consumer preferences for beer among German consumers, too. The majority (47.3 %) named Pilsner beer as the type of beer they drink most. Followed by wheat beer (24.6 %) as their most preferred type of beer [104]. Participants further mentioned a wide range of beer brands available on the German market. Despite a decline in consumption and production in 2022, Germany was still the largest beer producer in Europe with around 1.500 breweries, where more than half of them were microbreweries [105]. Within this study, it became obvious that consumers prefer brands from large scale breweries, but also have preferences for beer brands of small and medium sized breweries.

When considering acceptance ratings there were significant differences in overall liking, as well as flavour liking between the beers. In terms of aroma, none of the beers tasted were significantly preferred over the others. According to Gonzalez Viejo et al. (2019) aroma compounds leading to a higher liking (aroma, flavour and overall) are compounds showing fruity aromas (raspberry) and spicy/smoke/bacon aroma notes. Fruity compounds associated with apple, banana, or pineapple notes contribute to aroma liking and overall liking. Sweet, balsamic, and resin-like aroma and flavour are disliked by consumers. The authors identified styrene as a compound that causes these aroma notes by using an artificial neural network model [95]. Gender has an influence on flavour preferences in craft beer consumers. While men prefer beer with a more intense bitter taste, women prefer beer with lower levels of bitterness [102]. Therefore, it might be relevant to market beer brewed with an ancient spring barley variety with a higher level of bitterness towards male consumers, while beer brewed with ancient spring barley varieties and lower levels of bitterness should attract female consumers. It is relevant to communicate a beer's sensory profile to consumers, as consumers' expectations towards a beer, for instance, regarding its bitterness, influence consumers' liking of the respective beer [73].

It can be concluded that beer brewed with ancient spring barley varieties Mahndorfer Hanna and Kraffts Riedgerste are liked as much as a lager beer brewed with modern bred barley variety Avalon, while the aroma profile of the ancient spring barley Alpine Pfauengerste was less preferred, except for the creamier mouthfeel. So, it might be feasible to reduce the DMS and diacetyl content when brewing with this variety while still maintaining its creamy mouthfeel, which differentiates this variety from the others tested. The reduction of diacetyl depends on the pH-value of wort and beer, as well as temperature during fermentation [64]. At higher fermentation temperatures towards the end of the fermentation, the increased decarboxylation rate of α-acetolactate to diacetyl can be exploited with a so-called 'diacetyl-rest', resulting in a faster decrease of α-acetolactate concentrations in wort [106]. A lower wort pH (5.2) will be beneficial for diacetyl reduction. It is also recommended to avoid oxygen uptake post-pitching. Enough viable yeast cells during fermentation are also essential for diacetyl reduction. Rapid fermentation close to final attenuation is also beneficial [64]. The type of malt used also influences it. Malts kilned at lower temperatures contained higher concentrations of both valine and total amount of amino acids, leading to higher concentrations in wort [83]. Diacetyl formation in packaged beer can be reduced by the use of cool storage temperatures, limited oxygen presence, and sanitary practices [106]. Also malting practices can influence DMS. A higher moisture content, as well as higher germination temperatures, will lead to formation of DMS precursors, which can be reduced by higher kilning temperatures, especially when kilning for dark malt. Within this study, Pilsner malt was used, as the aim was to keep the barley varieties' sensory properties. Malting was also done as small-scale malting trials. So malting on larger scales and with adjusted malting parameters, might also improve malt quality. When working with ancient barley varieties, it might also be feasible to produce darker malts, with the risk of losing this varieties specific sensory profile. Kilning temperatures of 80 °C are supposed to reduce the DMS precursor content by 68 % [107]. As an infusion mashing procedure was used within this study, a higher DMS precursor content in wort can be expected. The application of decoction mashing would result in lower wort DMS precursor content. Wort boiling duration also affects DMS precursor degradation, and the intensity of boiling promotes the evaporation of free DMS. So longer boiling times, as well as higher boiling temperatures, would result in lower DMS content. The application of high-temperature boiling systems might also be feasible, as wort produced with this method is considered as being almost free of DMS [107]. Adjusting the malting and brewing process for Alpine Pfauengerste accordingly might result in lower lactic and cabbage-like aroma notes. For further studies, it is recommended to adjust the malting process for each variety individually, and to improve brewhouse performance of each ancient barley variety studied here and to achieve the best sensory profile of the final beer. Despite the fact that ancient barley variety Alpine Pfauengerste, did not perform well in brewing and in terms of consumer acceptance within this study, an adjusted malting and brewing process might increase consumer acceptance in further studies. Also, some effort should be made in testing different beer styles to determine which ones are more suitable for the varieties tested. Within this study consumer acceptance was solely tested on unfiltered lager beer, but other beer styles might be more suitable for these varieties. To avoid flavour alteration caused by a second fermentation in the bottle, it might be helpful to conduct further consumer acceptance testes with unfiltered lager beer which did not undergo a second fermentation. This might allow consumers to better perceive the flavour profile these ancient barley varieties might hold. It might also be recommended to use ancient barley varieties for brewing trials outside the German Purity Law, which gives brewers more flexibility to exploit these varieties' benefits. Nevertheless, ancient barley varieties are not suitable for large-scale industrial breweries. These varieties should be processed by craft beer brewers due to the higher workload and lower yields. The use of ancient barley varieties may contribute to the diversification of a breweries current product range and help increase customer interest, especially of consumers who are searching for new flavours and high-quality products. Beer brewed with ancient barley varieties should be advertised towards craft beer consumers, because they are often interested in trying something new and different [108]. Consumers nowadays also expect that higher quality beers show a specific flavour [109]. It might be feasible to inform and educate consumers about the distinct profile of ancient barley varieties, perhaps through beer tastings and marketing campaigns, as recommended by Williams and Baretta (2018). According to the authors, it is important to create memorable customer experiences through tastings and tours, supported by social media activities. Further craft beer breweries should focus on differentiating themselves from other breweries by "storytelling", for instance, by highlighting which specific beers they produce and the raw materials used [108].

Results of this study showed that participants were willing to pay higher prices if these beers were produced and processed locally with ancient barley varieties. Hart (2018) stated that craft beer consumers would pay 0.25–0.54 dollar more for a locally produced beer [110]. This might compensate higher production costs, when brewing with ancient spring barley varieties. Higher production costs are due to lower yields, a more time-consuming brewing process, smaller batches, and lower extract in the brewhouse, as well as higher raw material prices resulting from lower grain yields. Mikyška et al. (2022) showed that the yield of ancient barley varieties was 2–3 times lower compared to modern bred barley varieties [66]. Also, Tucker et al. (2024) found lower yields in ancient barley varieties [111]. However, this might be influenced by the fact that the varieties studied by Tucker et al. (2024) were not well adapted to the environment in which they were tested. Another challenging aspect might also be the higher protein content of ancient barley varieties [53, 66]. As malting of ancient barley varieties requires very small batch sizes, this aspect might also increase raw material prices, thereby increasing the final beer's production costs. Due to the higher production costs and smaller batch sizes, it might also be an option to use beers brewed with ancient barley varieties to extend a brewers existing product range with some speciality, premium beers brewed with ancient barley varieties to attract additional consumers. According to Lerro, et al. (2020) craft beer consumers who drink craft beer mainly in pubs, after dinner with friends and colleagues are willing to pay between 4.00 to 6.00 Euros for a 330 ml bottle of craft beer [101]. Also, Faganel and Rižnar (2023) showed that consumers are willing to pay higher prices for high-quality craft beer [112]. It is relevant to market beer brewed with ancient barley varieties as craft beer. Craft beer is chosen by consumers because it is perceived as being of higher quality than commercial beer, especially when organic raw materials are used in its production [24]. Based on the results of this study and following other studies, it is recommended to use ancient barley varieties for craft beer brewing. Craft beer consumers are characterised by higher household income and therefore higher purchasing power [6, 102], which might offset elevated production costs without compromising overall profitability.

When beer, brewed with ancient barley varieties, is advertised, it is important to state that it is produced locally, it is sustainable and that it helps towards biodiversity and environmental protection. This is in line with findings of other studies. According to Hart (2018), craft beer consumers prefer locally produced beer from independent breweries [110]. Also Lebok and Putz (2022) stated that consumers expect higher quality beers to be brewed locally and in a sustainable way [109]. Faganel and Rižnar (2023) reported the relevance of personal interaction between craft-beer brewers and their consumers, as well as the presence of craft-beer brewers at craft beer festivals [112]. Attributes relevant for craft beer consumers are taste, fermentation process, colour, certification, as well as country of origin, while packaging material, store promotion, as well as brand and prices are less relevant when choosing craft beer [101]. Consumers nowadays prefer handcrafted beers [113]. Regarding retailing, Williams and Baretta (2018) recommend educating their sales force with the aim of continuing the transfer of the company's and products' story further [108]. Faganel and Rižnar (2023) recommended direct sales for personal interaction with consumers, or e-commerce, so consumers can easily access their products [112].

Overall, the use of ancient spring barley varieties is not recommended for large industrial-scale breweries. It is more relevant to craft beer brewers who want to brew beer with a specific sensory profile that differs from classic beer types. The integration of ancient spring barley varieties Mahndorfer Hanna or Kraffts Riedgerste might also be recommended for craft beer breweries which are brewing outside of the German Purity Law, which might also give brewers more flexibility in utilising these varieties more efficiently and by creating more interesting tasting experiences. Especially as among younger demographics, the importance of beer being brewed in accordance with the German Purity Law has diminished [113]. Incorporating ancient barley varieties despite their low agronomic performance, as well as the challenges during processing, might be a viable option, as consumers nowadays are seeking beers with a more complex and distinctive sensory profile [114]. For the successful use of ancient barley varieties, it is also important to educate farmers on how to grow these ancient barley varieties, as Martin et al. (2010) recommend in their study on Bere, an ancient Scottish spring barley landrace that gained new interest for whisky production and baking [115]. Martin et al. (2023) further stated the great relevance of implementing a reliable supply chain to ensure successful commercialisation, which also includes the technical equipment at all stages of the production process [3]. Incorporating these ancient spring barley varieties requires further research in terms of adjusting the technological processes for each barley variety, which is already common in modern industrial malting, where process parameters are adjusted based on variety and harvest location. A well-adjusted malting and brewing process might reduce the challenges encountered within this study. It might also ensure a better exploitation of the varieties sensory profile, but might also have limitations. Incorporating ancient barley varieties requires dedication by maltsters and brewers and their willingness to invest time and energy in exploiting the potential of these varieties and thereby contributing to biodiversity conservation. Despite that, working with ancient barley varieties will still be a niche market, as production costs are higher across the value chain, requiring consumers with a higher willingness to pay [116]. Following the recommendations of Mikyška et al. (2022), ancient barley varieties are beneficial for small-scale breweries or regional breweries, which will help to extend their product portfolio [66].

5 Conclusion

In the small-scale brewing trials, conducted within this study, it became apparent that brewing with the ancient spring barley variety Alpine Pfauengerste resulted in challenges during solid-liquid separation and a high level of coagulated protein after the whirlpool rest. Overall, brewing with ancient spring barley varieties resulted in lower alcohol content and apparent attenuation compared to the modern bred spring barley variety Avalon, despite the application of a three-step infusion mashing process. When brewing with ancient spring barley varieties, mashing in directly at temperatures > 60 °C, as nowadays common with well-modified malt, is not feasible. Instead, the brewing process should be adjusted to reach higher yields. Further research is required to adjust the malting process to each barley varieties needs, for improved malt quality. It is also worth to determine whether a decoction mashing process might be applied to achieve better extract yields. It can be expected that brewing with ancient spring barley varieties might result in filtration problems, especially with Alpine Pfauengerste, which had a very high protein content. Within this research project, beers were not filtered and underwent a second fermentation in the bottle, which is not common practice in lager beer production. For further research it is recommended to work with filtered beer which did not undergo a second fermentation to avoid masking or altering flavour differences attributed to barley varieties. Based on the results of this study, it is these varieties are suitable for the production of unfiltered beer, as craft beer consumers, are increasingly open to drinking unfiltered beer. The results of this study are based on small-scale brewing trials so it is recommended to verify the results with other vintages and malt types. It would also be meaningful to investigate how ancient barley varieties respond to an upscale of the brewing process.

Further research is needed to study whether these varieties are suitable for other beer styles, as the focus of this study was solely on lager beer. Lager beer brewed with ancient spring barley varieties showed a slightly distinctive sensory profile compared to lager beer brewed with the modern spring barley variety Avalon. Lager beer brewed with ancient spring barley variety Alpine Pfauengerste showed a significantly creamier, less watery mouthfeel and a more intense colour than the other varieties tested. The colour of ancient spring barley varieties Mahndorfer Hanna and Kraffts Riedgerste resulted in a significantly more pale-yellow colour compared to the modern bred spring barley variety Avalon.

When comparing the sensory profile of hot steep malt extracts with the profile of the resulting final beer, it can be stated that the brewing process itself has a large influence on the aroma, flavour and mouthfeel of the final beer. For instance, the addition of hops as well as fermentation by-products change the beer's sensory profile. Hot Steep Malt Sensory Evaluation Method in combination with Check-all-that Apply Method is not able to predict the flavour of the final beer. These two methods together are very suitable for quality control and to test for off-notes in base malt, as they are easy to execute and require only a small amount of malt. Predicting from the malt's sensory profile the flavour of the final beer using the two methods together is challenging.

Lager beer brewed with ancient spring barley varieties Mahndorfer Hanna and Kraffts Riedgerste were liked as much by consumers as lager beer brewed with modern bred spring barley variety Avalon. In contrast, consumers liked lager beer brewers with ancient spring barley variety Alpine Pfauengerste less compared to the modern bred barley variety Avalon. However adjusted process parameters might also improve the quality and therefore the sensory profile of the final beer brewed with Alpine Pfauengerste. The majority of consumers stated that they were willing to support a concept where ancient barley varieties are grown, processed, and sold locally. Consumers were also willing to pay higher prices for such a beer. The majority of participants stated that they would pay, in a food retail store, up to0.50 Euro or even up to 1.00 Euro more per 0.5 L bottle of beer brewed with ancient spring barley varieties. Participants further stated that they value attributes such as sustainability and regional produce. So, it might be suitable to integrate those aspects into marketing campaigns.

Overall, it can be stated that ancient barley varieties are an ideal solution for craft beer brewers or local small-scale breweries who want to create a unique selling point by using ancient barley varieties, but not for large-scale industrial breweries. Ancient barley varieties have higher raw material prices and require a more time-consuming brewing process, which makes these varieties less interesting for standardised large-scale breweries, which have to focus on creating a standardised product and using a very efficient brewing process. Further research is needed to adjust the process parameters to each ancient barley variety to exploit each varieties full protentional. It might also be feasible to think about using ancient barley varieties for beer styles brewed outside of the German Purity Law, which gives brewers slightly more flexibility. This might be an option as craft beer consumers are more open to taste products with a new flavour profile, and as for younger consumer cohorts, where compliance with the German Purity Law is no longer a primary criterion for beer preference or purchasing decisions. Craft beer consumers perceive craft beer as being of higher quality and value the use of local ingredients. As they have a higher purchasing power, this might cover the higher production costs of beer brewed with ancient barley varieties.

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Conflicts of interest

The authors declare no conflict of interest.

Author contributions

Doris Häge: conceptualisation and methodology, formal analysis and validation, writing (draft and review), visualisation, project administration; Candy Ponickau: investigation, review; Bernd Lindemann: funding acquisition, supervision, review.

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117. Supplementary material
118. Table S1: Attribute list for descriptive analysis modified from [23, 61]
119. Table S1 cont.: Attribute list for descriptive analysis modified from [23, 61]
120. Table S1 cont.: Attribute list for descriptive analysis modified from [23, 61]
121. Figure S1: Check all that Apply Data – Aroma of Hot Steep Malt Extracts [53]
122. Figure S2: Check all that Apply Data – Taste of Hot Steep Malt Extracts [53]

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Supplemetary material

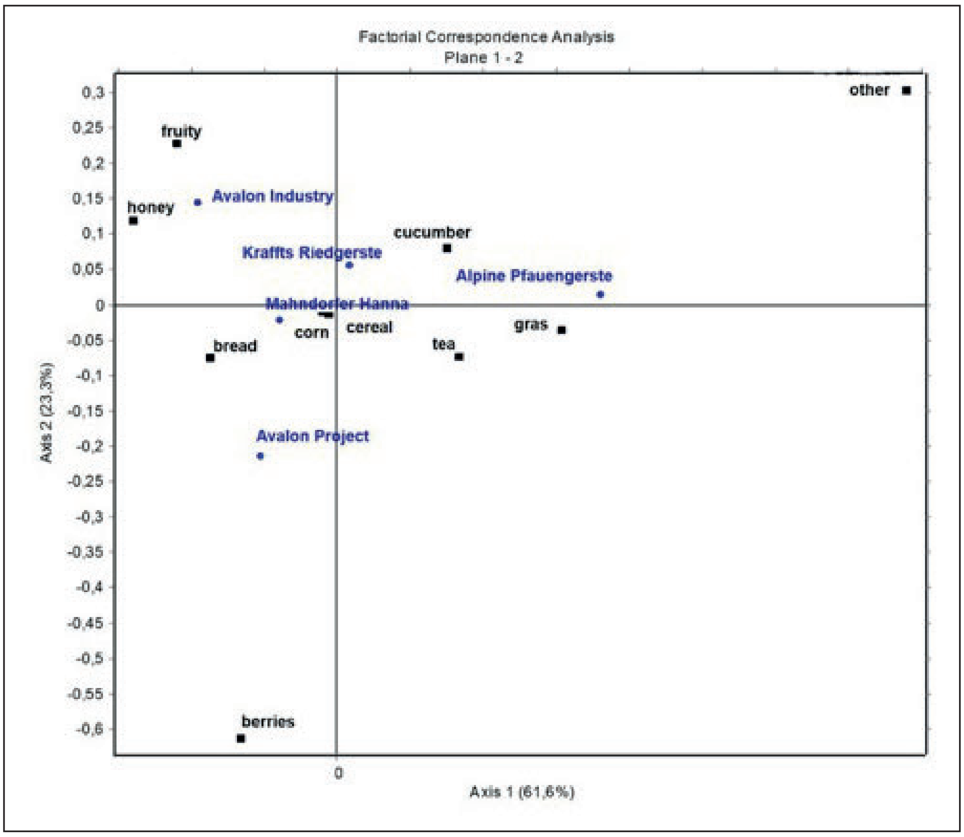

Fig. S1 Check all that apply data – aroma of hot steep malt extracts [53]

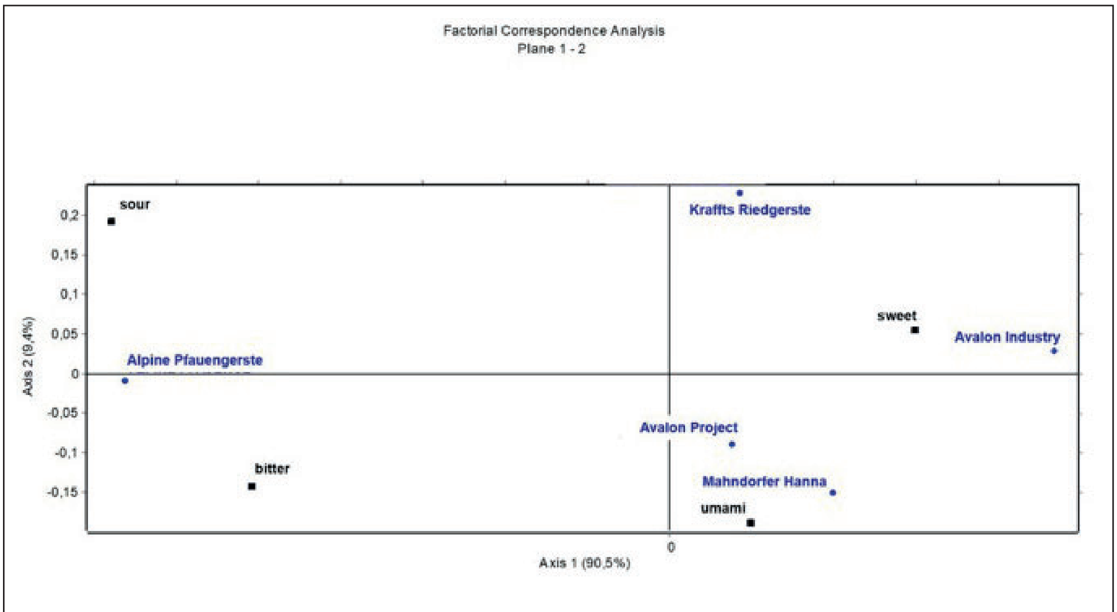

Fig. S2 Check all that apply data – taste of hot steep malt extracts [53]

Table S1 Attribute list for descriptive analysis modified from [23, 61]

Modality	Attribute	Description
Appearance	Beer turbidity (not perceivable – very intense)	Determination of how turbid the beer appears, from not perceivable to very intense
	Beer colour (pale yellow – brownish)	Determination of the beer colour from pale yellow to brownish
Aroma	Banana (not perceivable – very intense)	Reminiscent of ripe banana fruits
	Citrus (not perceivable – very intense)	Hop-like aromatics that remind of citrus fruits
	Berries (not perceivable – very intense)	Reminiscent of red berries like cassis
	Green apple (not perceivable – very intense)	Pungent aroma, reminiscent of green apple
	Floral (not perceivable – very intense)	Reminiscent of jasmine or rose petals
	Green-grass (not perceivable – very intense)	Reminiscent of fresh cut grass
	Cereal (not perceivable – very intense)	Reminiscent of grains or freshly ground cereals
	Bread (not perceivable – very intense)	Reminiscent of freshly baked and broken bread crumb
	Butter (not perceivable – very intense)	Reminiscent of fresh butter
	Cabbage (not perceivable – very intense)	Sulphur-like aroma compounds, reminiscent of cooked cabbage or vegetables
Flavour	Banana (not perceivable – very intense)	Characteristic taste of ripe bananas
	Citrus (not perceivable – very intense)	Taste sensation of citrus, reminiscent of hops
	Berries (not perceivable – very intense)	Taste sensation of red berries like cassis
	Malty (not perceivable – very intense)	Characteristic taste sensation of malt or malt extract
	Cereal (not perceivable – very intense)	Taste of grain or freshly ground cereals
	Bread (not perceivable – very intense)	Taste of freshly baked bread and broken bread crumb
	Butter (not perceivable – very intense)	Taste of fresh butter
	Bitter (not perceivable – very intense)	Bitter basic taste
	Sour (not perceivable – very intense)	Sour basic taste sensation, no milk sour taste
	Sweet (not perceivable – very intense)	Sweet basic taste sensation; taste of sucrose
Mouthfeel	Tingling (not perceivable – very intense)	Sensation of CO_2 released in the mouth
	Creamy (not perceivable – very intense)	Sensation where the beer feels full-bodied in the mouth
	Astringent (not perceivable – very intense)	Sensation caused by organic acids or polyphenolic compounds/tannins, no synonym for sour
	Coating (not perceivable – very intense)	Sensation of a thin layer that covers the surface of the mouth
	Watery (not perceivable – very intense)	Watery beer feels thin, no volume in the mouth
Aftertaste	Banana (not perceivable – very intense)	Aftertaste of ripe bananas
	Citrus (not perceivable – very intense)	Aftertaste of citrus, reminiscent of hops
	Bitter (not perceivable – very intense)	A bitter taste that lingers in the mouth after the sample has been swallowed.
	Sour (not perceivable – very intense)	A sour taste that lingers in the mouth after the sample has been swallowed. No milk sour aftertaste