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Key constituents, flavour profiles and specific sensory evaluation of wheat style non-alcoholic beers depending on their production method

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Analytical and sensory profiles of non-alcoholic beers (NAB) vary on the process used for their production. The style and production procedure with blending and subsequent aroma enhancement lead to a wide range of product specific characteristics, which have so far only been evaluated using attributes and schemes developed for standard alcoholic beers. There has been no comparison and characterisation of wheat style NABs from different production processes using olfactometry in combination with sensory analysis. GC-O/MS sniffing was performed to identify the aroma active components in wheat style NABs produced by different methods and the alcoholic standard beer to determine the differences in their aroma spectrum. Based on this, a sensory scheme for the targeted assessment of aroma profile, flavour intensity and attributes describing non-volatile properties was developed and validated using top fermented NABs. The odour activity of aroma substances differ depending on the matrix. The choice of attributes varies depending on the production process and aroma profiling is not always sufficient for the holistic characterisation of NABs. © 2021 The Authors. *Journal of the Institute of Brewing* published by John Wiley & Sons Ltd on behalf of The Institute of Brewing & Distilling.

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Keywords: non-alcoholic beer; thermal dealcoholisation; limited fermentation; GC-O/MS; panel training; sensory evaluation; aroma profile analysis

Introduction

In recent years, consumers' attitude towards non-alcoholic beers (NAB) has changed (1, 2). Papers have been published on the motivation as well as the functional and emotional associations (conceptualisation) behind consumers choosing NABs rather than their alcoholic counterpart (3, 4). People like the flavour of beer, but not the associated risk of inebriation and losing control. Nowadays, they are more than a substitute product and are consumed as an energy source, thirst quencher, for pleasure or relaxation (5, 6).

NABs are the fastest growing segment on the beer market and thus became attractive for breweries of all sizes and structures (1, 2). The process of producing NABs has a substantial influence on the beverage profile and composition. Depending on the methods used for dealcoholisation (i.e., physical), reduced ethanol formation (i.e., biological), or hybrid ones (i.e., blend) the beers differ in their volatile and non-volatile composition as well as their sensory perception (7–9). While an acidic taste is frequently criticised in dealcoholised beers, warty off-flavours and a sweet taste are typical of NABs produced by limited fermentation (10). Aroma characteristics in low alcohol beers and NABs have been noted in a variety of studies that compared single production methods. These have been summarised and discussed in several review articles (8, 9, 11). The utilisation of thermal dealcoholisation (TD) causes extensive loss of volatile aroma components. Similarly, membrane methods, which work at low temperatures and result in beers with a low aromatic profile and less body (10). The attribute 'body' or more precisely palate fullness is often mentioned as a deficiency of NABs and needs to be considered separately from the volatile aroma profile. The non-volatile matrix substances

(especially polysaccharides) influence the sweetness and palate fullness of cereal based beverages depending on their different macromolecular fractions (12). Beer brewed by limited fermentation (LF) is inharmonious in taste and has an immature aroma due to warty off-flavours, mostly caused by aldehydes (e.g., 2-methylbutanal, 3-methylbutanal, 2-phenylacetaldehyde) and a lack of fruity aroma due to limited yeast metabolism (13). Thus, the sensory evaluation within the product group is divergent and complex, especially for consumers.

Flavour terminology

The terms aroma, taste and flavour are defined as follows in this study. Aroma is the collective term for volatiles (i.e., free and bound odorous substances that are released when eating or drinking) that originate from a food or beverage as the orthonasal impression (14). Taste describes the gustatory perception (i.e., salty, sweet, sour, bitter, umami) caused by soluble substances (14). Flavour is the combined impression of perceived ortho- and retronasal (14).

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For this study, instrumental analysis evaluates the aroma, while sensory evaluation uses both olfactory and gustatory senses to describe the flavour. Trigeminal stimuli as elicited by ethanol and product carbonation are also considered with regards to a multimodal origin of sensory perception (15).

In the late 1970s, Meilgaard introduced a flavour terminology, that provides the basis for the current sensory standards for beer and malt-based beverages (16–19). This involves 14 classes, 44 first tier terms and 78 second tier terms to describe and define the identified flavours in beer. Only a limited number of attributes are suitable for the evaluation of NABs and, in a daily tasting routine, the complete list of attributes can easily fatigue panellists. The collection of sensory attributes for NABs and related beverages such as sodas, seltzers and mixed beverages show the high degree of diversification in the NABs product group (20, 21). Not only do the type of variety, production technique or yeast (*Saccharomyces*/non-*Saccharomyces*) lead to different characteristics (11), various pre- and post-processing steps result in a diverse product portfolio based on data obtained from consumer acceptance tests (5, 20–22).

The first customised list of 21 attributes for the sensory evaluation of NABs was published in 2013 (5). These attributes were described in four categories: (i) hop aroma and sweet, (ii) boiled cabbage-like, sour and bitter, (iii) malty, honey-like as well as sweet, and (iv) bitter plus mouth coating. More recently, Ramsey et al. (22) followed with a clustering of an 'overall liking' dataset into (i) malty, (ii) cooked vegetable, (iii) banana pear drop aroma, (iv) bland and (v) hoppy NABs. The results show the spectrum, deviation and different characteristics of the products. Nevertheless, a specific sensory scheme as well as a link to flavour components for NAB wheat beers have played a subordinate role and there has been no explanation which components are responsible for off-flavours in NABs.

The contribution of a flavour substance to the overall flavour impression can vary depending on the matrix. Piornos et al., (23) determined the thresholds for 26 flavour substances in a non-alcoholic beer model created to match a NAB brewed by a cold contact process or limited fermentation. They showed that the aroma intensity of the 26 selected compounds differ if they are measured in NAB or other matrices such as water, oil or alcoholic beer.

Up to now, the chemical, aroma-analytical and sensory evaluation of NABs has been carried out as an adaptation of the analyses used for regular alcoholic beers. For this reason, two approaches were developed in product development. On the one hand, there are breweries that try to modulate and design a beverage as close as possible to standard, alcoholic beers (24). Alternatively, breweries want to develop malt-based, 'standalone' beverages with balanced, positive organoleptic characteristics.

Knowledge about the aroma active substances is useful at the preliminary stage. These shape the profile of the beverage according to their threshold and concentration in the respective matrix. Extensive research has been carried out in the field of key aroma components for different beer styles (25–28), but it has not been transferred to a standard driven sensory evaluation.

The aim of this study is to evaluate the samples based on their chemical and sensory properties, compare the different NABs at one level and propose a sensory scheme that can be used to comprehensively evaluate NABs. The objective is the easy adaptation and use by panellists who are trained on beer and beer-based beverages. The aroma active substances of three different samples were analysed to show the differences between the production methods for NABs. In order to identify the influence of the

dealcoholisation method and the associated loss, retention or enrichment of aroma compounds during thermal dealcoholisation (i) the original alcoholic beer (beer WO) before dealcoholisation and (ii) the dealcoholised equivalent NAB-TD were analysed. A third sample (iii), a NAB produced by limited fermentation (NAB-LF) was also included. The base product of the NAB-LF is wort, which is free from fermentation by-products and was not considered for the aroma analysis. Knowing the aroma active compounds determined via aroma dilution analysis ensures that product specifics can be determined according to their production or any pre and postproduction process. Building upon this, a useful choice and structure of descriptors was carried out to design a sensory scheme for the volatile profile and non-volatile matrix for different NAB styles. Finally, the application of the compiled scheme for the two NABs analysed via aroma dilution analysis, is reported.

Materials and methods

Beer and NABs for instrumental analysis.

For instrumental aroma analysis a 10 hL batch of top fermented wheat beer (5.31% ABV, beer WO) was thermally dealcoholised in the Research Brewery Weihenstephan and dealcoholised to 0.05% ABV (sample NAB-TD) in the rectification column (Dealcotec[®], Centec GmbH, Maintal, Germany) of the Institute of Brewing and Beverage Technology, TUM School of Life Sciences, Freising, Germany. A commercially available non-alcoholic wheat beer produced via limited fermentation (sample NAB-LF, 0.29% ABV) was included for comparison purposes.

Panel training. Commercially available NABs (n = 23) were purchased for panel training sessions. Ten top fermented wheat beers and 13 bottom fermented samples (e.g. pilsner and lager) were used. For training purposes, top and bottom fermented beers were selected to demonstrate the differences between the varieties. As it is common to blend beers produced by biological and physical methods in variable proportions, six of the samples were blended.

Determination of aroma classes in the sensory scheme. Clustering of the aroma classes was carried out with the ten top fermented samples from the panel training. Physicochemical analysis data and corresponding methods are listed in Table 1. The validation of the resulting scheme was performed with NAB-TD and NAB-LF.

Part 1: Aroma analysis

The aroma spectrum was characterised according to the approved standard methods of the Central European Commission for Brewing Analysis (MEBAK) (29). All analyses were conducted in duplicate. A gas chromatograph coupled with a flame ionisation detector (GC-FID) was used for quantification of beer aroma compounds (MEBAK 2.21.2.2) as well as higher alcohols and esters (MEBAK 2.21.6). Ethanol (purity 99.5% (v/v)) was added to the NAB sample to adjust the ethanol content to 10% (v/v) (MEBAK 2.21.2.2) and 5% (v/v) (MEBAK 2.21.6), respectively. As a key contributor to the flavour in alcoholic wheat beers (27), the non-volatile compound, 4-vinylguaiacol (4-VG) (2-methoxy-4-vinylphenol) was measured using high-performance liquid chromatography (HPLC) coupled with a diode array detector (DAD) following the MEBAK 2.21.3.3 method (29).

Table 1. Physicochemical characteristics of the German top fermented wheat beer samples used for the NAB panel training, PCA and clustering analysis

Samples	Production method	Original gravity [% mass]	Ethanol [% (v/v)]	Real extract [% mass]	pH	Bittering units [IBU]	Osmolality [mOsmol/kg]	CO ₂ [g/L]
Method		MEBAK 2.9.6.3	MEBAK 2.9.6.3	MEBAK 2.9.6.3	MEBAK 2.13	MEBAK 2.17.1	MEBAK 2.10.2	Lab.com, ACM
1	LimFer1	7.26	0.36	6.71	4.36	20	272	6.8
2	LimFer2	7.16	0.29	6.72	4.67	14	276	5.5
3	LimFer3	7.26	0.19	7.34	4.49	15	263	6.3
4	LimFer4	7.33	0.30	6.99	4.56	17	304	6.2
5	Blend1	6.27	0.48	5.53	4.26	19	239	5.6
6	Blend2	6.71	0.43	6.04	4.44	21	264	4.8
7	Therm1	5.18	0.44	4.49	4.27	15	215	6.2
8	Therm2	4.75	0.34	4.21	4.28	12	185	6.0
9	Therm3	5.81	0.37	5.24	4.37	20	217	6.1
10	Therm4	7.25	0.43	6.60	4.27	15	257	6.5

'Therm' describes thermally dealcoholised beers, 'LimFer' beers produced via limited fermentation and 'Blend' a blend of both. The samples were packaged in 0.5 L amber glass bottles (NRW and swing top). The mean of three technical replicates is reported.

GC-O/MS for aroma profile and dilution analysis. Headspace solid-phase microextraction (HS-SPME) coupled to GC-olfactometry/mass spectrometry (GC-O/MS) was used to determine the aroma activity of the volatile fraction of the samples. This was done in accordance with previous literature (30, 31) with the only difference that a HS-SPME was performed without solvent-assisted flavour evaporation (SAFE) due to interfering substances in the medium of NABs.

Sample preparation for HS-SPME. Unfiltered sample (5 mL) was added to a 20 mL headspace vial and incubated at 40°C for 10 min. HS-SPME was carried out for 30 min at 40°C with a DVB-CAR-PDMS fibre (Stable Flex, 50/30 µm, 24 Ga, Supelco Inc., Bellefonte, PA, USA). After adsorption, the fibre was transferred directly to the injection port of the gas chromatograph.

GC-olfactometry-MS parameters. Analysis of the SPME fibre was carried out on a Trace 1300 Gas Chromatograph (Thermo Fischer Scientific Inc., Waltham, MA, USA) equipped with a TG-5MS column (Thermo Fischer Scientific Inc., Waltham, MA, USA; length: 60 m, inner diameter: 0.25 mm, film thickness: 0.25 µm). The flow rate of the carrier gas, helium, was 1.85 mL/min. The injector temperature was 250°C and the transfer line temperature 200°C. The oven program started with an initial temperature of 60°C and was held for 4 min. Subsequently, the heating rate was 5°C/min until a final temperature of 250°C and this was then held for 3 min. The GC was coupled to a single quadrupole mass spectrometer (ISQ QD, Thermo Fischer Scientific Inc., Waltham, MA, USA) and an olfactory detection port (ODP 3, Gerstel, Mühlheim an der Ruhr, Germany). The effluent was split into two equal parts with a micro-flow splitter. The sniffing port was heated to 250°C and flushed with humidified air to avoid any dehydration of the nasal membranes of assessors. MS detection was performed with an electron impact (EI) energy of 70 eV. The analysed mass range was 35–350 amu. Peak detection was performed in a Thermo Xcalibur 3.1.66.10 (Thermo Scientific Inc., Waltham, MA, USA).

Aroma compound identification. The identification of aroma compounds was based on odour description, linear retention indices (RIs), comparison to reference substances and mass

spectrometric data from the literature and NIST library. Linear RIs were determined after van Den Dool and Kratz using a mixture of linear alkanes C₆–C₂₀ under the same chromatographic conditions described above (32).

Aroma dilution analysis. To simulate a dilution series, the SPME fibre was injected into the gas chromatograph by different split modes, starting with the splitless mode and followed by split ratios: 1:3, 1:5, 1:10, 1:20, and 1:40. The flavour dilution (FD) factor for a particular compound is defined as the highest dilution at which that compound can be perceived by GC-O (33). This is the ratio of the concentration of the odorant in the initial extract to its concentration in the most diluted extract in which the odour is still detectable in the sniffing port (34). In this semi-quantitative analysis, the splitless run was used as the FD factor of one. The FD factor values of the other odorants were calculated on this basis and is a relative measure to identify the most aroma active substances in samples. Results of three assessors were used.

Part 2: Panel training and developing a specific sensory scheme

The sensory panel consisted of 14 members from the Institute of Brewing and Beverage Technology who were trained weekly to objectively and adequately replicate the characterisation of NABs. All panellists were experienced and had been trained with previous sensory evaluations of beer, craft beer and beer-based mixed beverages according to DLG quality test guidelines (35). The training in this study focused specifically on the different NAB styles.

The panel training was performed according to the sensory standard guidelines (17, 19, 36) as a modified quantitative descriptive analysis. Up to five samples of one beer style were included per session. In all sessions, the panellists were given 50 mL of the sample. All samples were provided with a random numerical code, at a temperature of 12°C, and were poured without foam. The CO₂ content varied due to the production process and the measured CO₂ content was typical of the style and ranged from 5.3–6.5 g/L.

The following aspects were the focus of the training: (i) the use of specific attributes to describe NABs, (ii) calibration of the panel

to NAB attributes, (iii) direct comparison of product samples, and (iv) classification of product samples in the entire spectrum.

For panel training, lager beer was used, as the tasters are more used to these. It was therefore easier for them to differentiate and characterise the wheat beer NABs. The first step was to generate a vocabulary and descriptors that accurately differentiate the samples and NAB styles. A consensus profile with reference to published literature was used at this stage for attribute determination (16, 19, 37, 38). Term lists for the aroma evaluation were created using the generated terms and descriptors. Similar terms were combined, and hedonic attributes eliminated in these lists. The individual descriptions were summarised in a group protocol and checked for their frequency distribution according to MEBAK guidelines (18).

In addition, each panellist was asked to provide supplementary terms while assessing the samples. Once the NAB term pool was generated, the first step in scope of the panel training and designing a sensory evaluation scheme specific for NABs was intensive discussion, reduction of the number of attributes to be included in the evaluation scheme and subsequently scale training/intensity measurements of the attributes. The panellists were asked to evaluate the intensity of the most cited (absolute frequency, relative frequency) aroma attributes on a five-point, linear interval scale ranging from 0 (not detectable) to 4 (very intense). In addition to aroma profiling during panel training, additional attributes were compiled and queried to evaluate a more specific description of the beer matrix using a five-point 'Just-About-Right' (JAR) ordinal scale, which are used in consumer acceptance tests (39).

Data analysis. Tasting sessions were conducted with the computer-aided sensory analysis tool FIZZ, version 2.60.00.1512 (Biosystèmes, Couternon, France). Statistical analyses, including two-sided cluster analysis and principal component analysis (PCA) were performed using JMP[®] Pro (Version 14.1.0., SAS Institute, Cary, NC, USA). OriginPro 2020, version 9.7.0.188 (OriginLab Corporation, Northampton, MA, USA) was used for figures.

Results and discussion

The first part of this study concerned the quantification of typical aroma substances in beer via GC-FID and the identification of aroma active components via GC-O/MS of three wheat beer samples. The aim was to determine the difference between (i) alcoholic and non-alcoholic beers and (ii) NABs from different production methods. The collected data helped determine the impact of the production process on the aroma active compounds in the samples. Furthermore, it generated a preliminary pool of terms to be used in the development of the NAB tasting scheme. The advantage of the attributes selected from the chemical data and instrumental measurements is that these terms fulfil the requirements for sensory evaluation tailored to NABs. Moreover, the chemical data confirms whether the perceived compounds are aroma compounds or off-flavours related to the production process of the NAB. From the sensory data collected in the panel training sessions, it was possible to select the most accurate descriptors for the volatile (e.g. estery, worty) and non-volatile matrix (e.g. watery, sweet, carbonation, bitterness), group the terms into categories (e.g. fruity, cereal, and the umbrella-term 'palate fullness') and validate it using statistical analyses. Finally, appropriate scales that discriminate between the characteristics were selected.

Aroma components in in wheat beer WO and NABs

The concentrations of typical beer aroma substances were measured in three representative wheat beer samples and the analytical differences determined. The volatile fraction of these samples was then characterised using GC-O/MS and the aroma active substances identified. Finally, attributes for their profile description were selected for each sample from the collected data.

Figure 1 shows the concentrations of the aroma compounds measured using GC-FID for higher alcohols, acetate esters, fatty acid esters, fatty acids and hop-derived aroma components. HPLC was used to quantify 4-vinylguaiacol. The concentration of the aroma compounds in NABs is significantly lower compared to beer WO, regardless of the method (biological or physical) used for production. Our data is in agreement with previous studies (9), where single samples or one production method were analysed. It further shows that the three samples, beer WO (reference beer), NAB-TD (physical method) and NAB-LF (biological method) should be considered and evaluated independently.

During thermal dealcoholisation, most volatile fermentation by-products (i.e. higher alcohols, esters, and hop-derived aroma components) evaporate with ethanol and are accordingly present at lower concentrations in NAB-TD. The concentration of acetaldehyde was reduced by 78% in NAB-TD compared to beer WO, 3-methylbutyl acetate by 98% and geraniol by 96%. Due to their low volatility, 2-phenylethanol (rose-like), the phenolic compound 4-VG and the fatty acid fraction were not lost during the physical separation process.

The impact of both production processes was compared on the aroma fingerprint of NAB-TD and NAB-LF. Although the raw materials were not identical, NAB-LF was chosen to make a standard comparison of the aroma compounds as, in general, the measured values are lower in beer brewed by a biological process. The lower concentration of higher alcohols and 4-VG is due to limited yeast metabolism. Similar behaviour is observed for the total of acetic acid esters and fatty acid esters. The concentration of medium-chain fatty acids, whose content depends on the selection and composition of raw materials, are lower in NAB-LF. The concentration of hop-derived aroma components in NAB-LF is also lower. This depends on the hop treatment (variety, quantity, boiling time) used in the production process, which is comparatively low for wheat-type NABs. In comparison to lager or pilsner type NABs (20). Only 3-methylbutanoic acid (isovaleric acid), which is associated with oxidised hops and/or yeast autolysis (18), is higher in NAB-LF than in the thermally dealcoholised sample NAB-TD.

Identification of aroma active compounds via SPME and aroma dilution analysis.

In addition to the chemical data discussed above, an aroma dilution analysis was performed with the wheat style beer WO and NABs. GC-O/MS was used to identify the aroma active compounds and determine their individual contribution to the overall aroma. Table 2 shows the volatile substances, odour qualities and retention indices (RI) of the beer WO, the resultant NAB-TD, and NAB-LF. It also lists the FD factor of each aroma substance. In accordance with Langos et al. (27), volatiles, although detectable in high concentrations did not result in an odour impression at the sniffing port, whereas others smelled or were sensorially active even though they did not show a signal, due to different odour thresholds of the aroma substances in water or beer. Aroma compounds formed during fermentation, such as ethyl butanoate (FD = 20) and ethyl hexanoate (FD = 20), had the highest FD factors in the original beer WO. 4-VG is a characteristic aroma and flavour substance in phenolic wheat beers that

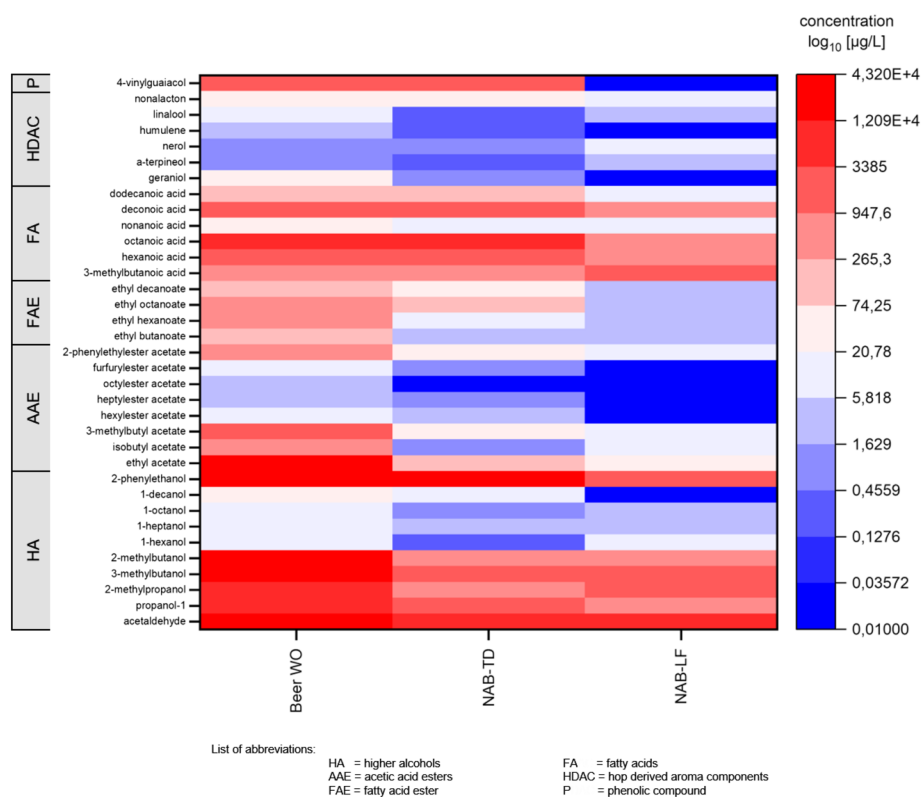


Figure 1. Heatmap of the aroma components concentration of the original, alcoholic beer (beer WO), the NAB after thermal dealcoholisation (NAB-TD) and the non-alcoholic beer obtained by limited fermentation (NAB-LF) on a logarithmic scale. The analysis of the beer aroma components was made according to MEBAK W.B.B.M. 2.23.6., MEBAK W.B.B.M. 2.21.6 and MEBAK W.B.B.M. 2.21.3.3 (MEBAK, 2012). [Colour figure can be viewed at wileyonlinelibrary.com]

also scored a FD factor of 20. Components that depend strongly on the hop variety, quantity and treatment can be qualitatively verified in the beer WO and the corresponding thermally dealcoholised beer NAB-TD (Table 2). These include linalool, DMTS (dimethyl trisulfide), and the highly aroma active ketone (*E*)- β -damascenone, which is a glycosidically-bound compound (40).

In this study, HS-SPME was used to analyse the volatile fraction instead of solvent assisted flavour evaporation (SAFE). However, the identified key aroma components in alcoholic wheat beer are in agreement with previously published data (27, 41). Ten aroma compounds in the beer WO have a FD-factor of 20 and contribute strongly to the characteristic wheat beer aroma.

Hop-derived aroma substances such as DMTS (cooked cabbage) and (*E*)- β -damascenone, with an odour of cooked apple (27) or cherries, survive dealcoholisation. The monoterpene linalool is partly evaporated during the process and is only detectable in NAB-TD in the splitless run. Acetic acid is aroma active in NAB-TD (FD = 5), but not in the reference beer WO, suggesting it is enriched and emerges in the NAB after thermal treatment. 2-Phenylethanol (rose-like) and 4-VG are also enriched during dealcoholisation. These substances scored higher FD factors in NAB-TD than beer WO. Of the ten aroma substances with an FD factor of 20 in the beer WO, only 4-VG and (*E*)- β -damascenone can be detected in the resulting NAB-TD with a similar or higher FD factor. As previously noted, phenolic substances (e.g. guaiacol, vinylphenol, 4-VG) are less volatile than fermentation by-products and therefore survive thermal dealcoholisation. From the sensory perspective the ortho- and retronasal thresholds of 4-VG in NABs are lower compared to standard beer (23), which supports the hypothesis

that single aroma substances in NABs are more odour active than in alcoholic beers. In an alcoholic matrix, the number and higher concentrations of other aroma compounds mask them.

The aroma dilution analysis was also carried out for NAB-LF. The high FD-factor (40) for linalool indicates a late and/or high addition of hops during processing in the brewhouse. The hop treatment (variety, quantity, time) differs to that for beer WO and NAB-TD as shown by the lack of (*E*)- β -damascenone, which is not one of the aroma active substances in NAB-LF. Although raw material selection for NAB-TD and NAB-LF are not identical, the collected GC-O/MS data suggest that the production process has a large influence on the aroma fingerprint of NABs (20, 22). Fermentation by-products can be detected, but the number and the FD factors are significantly lower in NAB-LF than in alcoholic beer. The high content of carbonyl compounds and lower degree of fermentation confirms that this beer was subjected to limited fermentation.

The fatty acid octanoic acid is aroma active in NAB-TD and NAB-LF, but not in the alcoholic beer WO. It is present and quantitatively detectable in beer WO (Figure 1) (beer WO, 6178 $\mu\text{g/L}$ vs. NAB-TD, 6054 $\mu\text{g/L}$ vs. NAB-LF, 752 $\mu\text{g/L}$) but not aroma active in the alcoholic matrix. A similar situation could apply to acetic acid. Indeed, it is assumed that the greater number and concentration of aromatic fermentation by-products mask other substances that are perceived as unpleasant in alcoholic beers. This suggests that off-flavours should be absent in the production of NABs, whether using physical or biological processes. They are in this case mid-chain fatty acids, or may already be other off-flavours which are aroma active in NABs at lower concentrations compared to alcoholic beers.

Table 2. odour-active volatiles of the original, alcoholic top fermented beer (beer WO), the thermally dealcoholised non-alcoholic beer (NAB-TD) and the non-alcoholic beer produced by limited fermentation (NAB-LF)

No.	Odour active compound	Odour quality	RI ^b	FD factor		
				beer WO	NAB-TD	NAB-LF
1	acetic acid	acidic	579	-	5	-
2	3-methylbutanal	caramel, cheesy, rancid	644	20	-	5
3	3-methylbutanol	malty	730	1	-	-
4	ethyl 2-methylpropanoate	sweet, fruity	755	20	-	10
5	ethyl butanoate	sweet, fruity	800	20	1	1
6	3-methyl-2-butene-1-thiol	skunk	826	10	1	3
7	3-methylbutanoic acid	rancid	834	1	5	1
8	ethyl 2-methylbutanoate	fruity, floral, berry	849	20	-	10
9	ethyl 3-methylbutanoate	fruity	852	5	-	-
10	ni ^c	plastic, musty	873	-	1	-
11	3-methylbutyl acetate	banana	876	5	-	5
12	styrene	solvent	897	-	-	3
13	2-furfuryl ethyl ether	sweet	904	-	1	-
14	methional	cooked potato	909	1	5	5
15	ethyl 4-methylpentanoate	fruity, peach	965	1	-	-
16	dimethyl trisulfide	cooked cabbage, stinkhorn	978	20	10	10
17	1-octen-3-ol	mushroom	979	1	1	-
18	ethyl hexanoate	fruity, strawberry	998	20	5	3
19	2-phenylethanal	honey sweet, floral	1049	-	1	5
20	furaneol	caramel	1058	5	1	1
21	1-octanol	musty	1071	-	-	1
22	ethyl-dimethylpyrazine	grain	1081	-	-	1
23	guaiacol	spicy, smoky	1093	1	-	3
24	linalool	floral, citrus-like	1098	20	1	40
25	2-phenylethanol	rose-like	1117	5	10	5
26	methionyl acetate	mushrooms, musty	1124	1	1	-
27	(E)-2-nonenal	cucumber	1162	-	-	1
28	ni ^c	rubber, dinghy	1184	-	1	1
29	octanoic acid	caprylic, goat	1184	-	1	1
30	ethyl octanoate	fruity, glue	1197	1	1	-
31	vinylphenol	dried fruits	1219	5	1	-
32	phenylethyl acetate	floral	1262	20	1	1
33	ni ^c	green banana, pungent	1301	-	1	1
34	2-aminoacetophenon	rancid	1315	1	1	-
35	4-vinylguaiacol	clove-like	1326	20	40	-
36	ethyl 3-phenylpropanoate	coconut	1358	5	1	-
37	(E)- β -damascenone	fruity, cherry	1396	20	20	-

Substance and odour quality perceived at the sniffing port and flavour dilution (FD) factors in ascending order of retention indices (RI).

^a odour quality perceived at the sniffing port by three panellists;

^b = RIs on a DB-5 column;

^c ni = not identified

It can be concluded that aroma active substances in NABs differ from alcoholic beers, the difference depending on the production method. Aroma substances with a lower volatility are retained by thermal dealcoholisation, carbonyls such as 2-phenylacetaldehyde emerge in beers produced via limited fermentation, and the number and concentration of aroma substances are significantly lower and present in other ratios (fermentation by-products of fatty acids) than in alcoholic beers. Substances that are described as having a negative impact on

the aroma in NABs, do not necessarily form during the dealcoholisation process. They may be masked in an alcoholic standard beer by fruity, sweet aromatic esters and only become perceptible in NABs, leading to an unpleasant aroma. If the aroma active compounds determined via aroma dilution analysis are known, product specific flavours and off-flavours can be determined according to their production method and be used for panel training. It is possible to generate terms and to select attributes for the evaluation scheme to be developed subsequently.

Structure of an evaluation of wheat style NABs

The results of the instrumental analysis confirm that the samples differ in their aroma profile (beer WO vs. NAB-TD and NAB-TD vs. NAB-LF) and suggest that the sensory assessments also differ considerably. The sensory characterisation of NABs requires a specific selection of attributes to adequately describe quantitative differences between the samples. Moreover, due to the significantly lower concentration of aroma substances in NABs compared to alcoholic beers, the sensory intensity scales require to be adjusted to accurately identify the quantitative differences between the samples.

Aroma classes of the descriptive scheme

Multivariate data analyses, i.e., principal component analysis and a two-sided agglomerative clustering analysis of the ten commercial wheat style NABs (see Table 1), were performed for the purpose of mapping and a data-based structuring of the individual attributes into aroma classes.

The result of the PCA (supplementary information, Figure S1) indicates the diversity of the individual samples in the score plot and describes 81.2% of the variation in the data (component 1: 61.2%, component 2: 20%). The similarity and relationship among the descriptors for the class selection can be discerned in the loading plot. As reported in studies for lager NABs (20, 22), the diversity within the product group is also evident for top fermented NABs. The hierarchical cluster analysis (Figure 2) shows the dendrogram of attributes (columns) and wheat beer NAB samples (rows). The abbreviation 'Therm' is used for the thermal dealcoholised beers, 'LimFer' for beers produced by limited fermentation and 'Blend' for a mixture of both. All of the columns have to be measured on the same scale, and is why only the continuous intensity scale is considered for clustering. The results of this analysis show a classification in the aroma categories 'estery', 'phenolic', 'floral' and 'acidic' (stem 1), 'cereal', 'worty', 'honey', 'sweet', 'bready' and 'caramel' (stem 2), and 'rancid', 'cardboard' and 'cooked potato' in stem 3. This grouping has been adopted for the scheme, with the

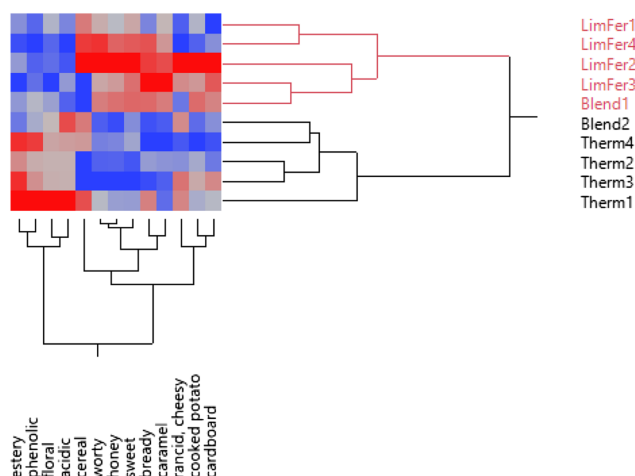


Figure 2. Dendrogram and colour coded graph of two-side hierarchical clustering analysis. Ten top-fermented commercial wheat style NABs and the attributes of the aroma profile are shown. The colour scale is given below; a diverging colour scheme from blue to grey to red for the intensities was chosen. [Colour figure can be viewed at wileyonlinelibrary.com]

exception that the attributes 'phenolic' and 'sour', which are strongly dependent on the beer style, are dealt with in a fourth group.

The choice of commercial NABs used for the study determine the results of the statistical analysis. Not all commercially available wheat style NABs are described by these clusters. This is why all classes have an extra line for free choice profiling, where additional descriptors can be included if required, as a toolbox. This blank space provides flexibility, which will allow the tasting scheme to be tailored to meet the requirements of the beer style (see Figure 3).

Grouping according to the cluster analysis and PCA, lead to the following aroma classes. Class 1 includes fruity fermentation by-products and fragrant hop aromas and is exemplified by 'estery' and 'floral' notes, as shown in the aroma dilution analysis (Table 2). Less fermentation by-products are present in NABs and these are less aroma active than in the alcoholic beer WO. Hop derived aroma properties are influenced by additional factors such as the style and the post production processes such as dry hopping used for the production of NABs (28).

Class 2 focuses on worty flavours; these are more intensive in NABs produced by limited fermentation. Their perception can vary from 'malty', 'honey', 'caramel' to 'cereal'. For dark beers, 'roasted' and 'chocolate-like' notes can be included and evaluated in the additional line of this class. In commercial NABs, which use blending for their production, the intensity of these attributes will depend on the ratio by volume of limited fermentation to thermal dealcoholisation.

Selected aroma compounds are considered as off-flavours when they are detected over a particular level in beer. Some (e.g. cardboard) are unacceptable regardless of concentration or beer style. Class 3 takes into account aromas that may already be present in the original beer and only become prominent due to the absence of the masking effect of aromatic fermentation by-products. Attributes associated with the ageing of beers (e.g. 'oxidation', 'bready') or flaws in production (e.g. 'diacetyl') are included in this group.

In class 4, beer style specific aroma notes can be evaluated. These include acidity, which can be divided into 'acidic', 'acetic' and 'lactic'. These aroma notes may not be simply process related due to the dealcoholisation method used, but are also particularly relevant since special yeast strains (e.g. *Cyberlindnera* yeasts (42), *Lachancea fermentati* (43)) can be used in the production of NABs. In addition, sour or fruit-sour beers have evolved as a type of NABs (44). The phenolic category is particularly relevant for top fermented NABs and should be used when evaluating top fermented wheat NABs. The phenolic note can be further described by a 'clove-like' aroma.

Additional classes of the descriptive scheme

Four additional classes for non-volatile properties are included in the scheme, as the panel training showed that evaluation is useful in addition to scoring on interval scales. In fact, ordinal scales are normally used for consumer acceptance tests (39). They can be applied as an option in this study and should be understood as a supplement to the aroma profile, allowing a more style specific description of the sample. Sweetness and acidity peaks, for example, which are evaluated on single scales during aroma profiling, can lead to a diminishing or lagging taste which is hard to evaluate using interval scales, but which affects the overall impression. This is why classes 5-8, in contrast to classes 1-4 are rated on JAR scales.

Extended scheme for the evaluation of non-alcoholic beers
Please mark the intensity of the attributes in class 1-4 and the assessment on the JAR scales in class 5-8



Figure 3. 'Extended scheme for NABs' developed using top fermented, non-alcoholic wheat beers as an example. [Colour figure can be viewed at wileyonlinelibrary.com]

Class 5 comprises a particularly complex category in the evaluation scheme and is given the umbrella term of 'palate fullness'. This class is challenging since a high score is not necessarily conducive to good quality and either extreme of the scale is undesirable. In NABs, a watery perception is not welcome, nor is a viscous and thick mouthfeel. A distinction is made between the evaluation of the attribute 'body' (i.e. palate fullness) assessed on a JAR-scale and a more detailed description of the mouthfeel in class 5. The former attribute should be included in the scheme and is rated from 'watery' to 'viscous, full-bodied'. The second attribute can be included as an option. 'Watery', 'smooth', 'slimy' or 'mouth coating' can be used as descriptors.

Class 6 assesses the 'harmony'. This term refers to the balance between sweetness and acidity. Similar to palate fullness, neither end of the JAR-scale (1 = acidity persistent; 5 = sweetness persistent) is desirable. NABs can be sweeter than alcoholic beers, due to the lower degree of fermentation, or more acidic,

which is process related. These two points are often discussed regarding the quality of NABs and consumer acceptance (5, 24, 45). A persistently sweet taste can have a negative effect on the drinkability of the beverage (46). NABs do not typically have a lower pH or a higher concentration of acidic substances. The acidic taste in alcoholic beers can be masked by the number and concentration of aroma compounds, but in NABs it can be perceivable. Whereas the attribute 'sweet' in class 2 and class 4 ('acidic/lactic') is evaluated according to the flavour intensity, the focus in class 6 is on the balance and long lasting final taste.

Carbonation is also one of the defining features of beer. The carbonation may be a little higher in non-alcoholic products to evoke a refreshing character or to mask off-flavours. Class 7 assesses the 'carbonation' level in NABs. The scale ranges from 'little effervescent' to 'excessively carbonated'. A NAB with a higher level of effervescence is preferred over a flat one (20).

The final class in the scheme is 'bitterness' (i.e. class 8) with the descriptors and terms used for this attribute can be extremely detailed (47). A distinction is therefore made between the JAR-scale evaluation, ranging from 'bitterness hardly perceptible' to 'excessively bitter' and the specific bitterness profile. In this class, a descriptor can optionally be included for the perceived bitterness profile. This is a detailed characterisation of to time-intensity responses produced by the bitter compounds in NABs; these profiles can be 'rounded', 'harsh', or 'progressive'. Following the classification shown above, Figure 3 illustrates the evaluation scheme developed in this study with the optional supplementation of the additional attributes.

Application of the scheme

To complement the chemical data in the first part of the study, and to demonstrate the discriminatory power of this scheme, the non-alcoholic wheat beers NAB TD and NAB LF, were assessed by the same tasting panel used for the proposed scheme.

Figure 4 diagrammatically compares the sensory aroma profile (class 1-4) of NAB TD and NAB LF. ANOVA shows significant differences in single attributes of the aroma profile of both NABs. Estery notes in class 1 scored higher in NAB LF ($\bar{x} = 1.43$) than in NAB TD ($\bar{x} = 0.75$). The biggest difference was observed in class 2 with regard to 'worty' ($p < 0.001^{***}$), 'bready' ($p = 0.0079^{**}$), 'sweet'

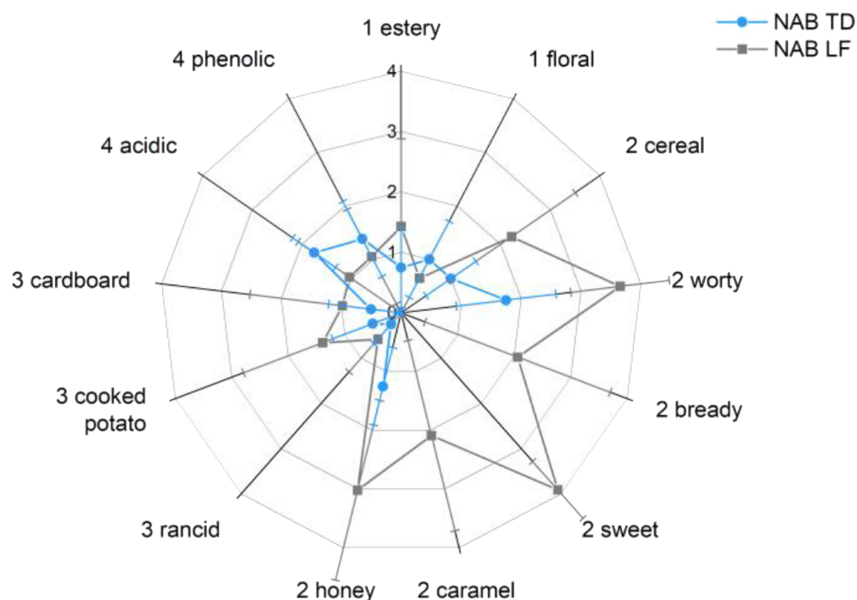


Figure 4. Intensities of the specific attributes for NAB TD and NAB LF according to the compiled aroma classes (mean \pm sd) on a five point linear scale from 0 (not perceivable) - 4 (very strong). The number added to the descriptor terms indicate the aroma class. [Colour figure can be viewed at wileyonlinelibrary.com] [Colour figure can be viewed at wileyonlinelibrary.com]

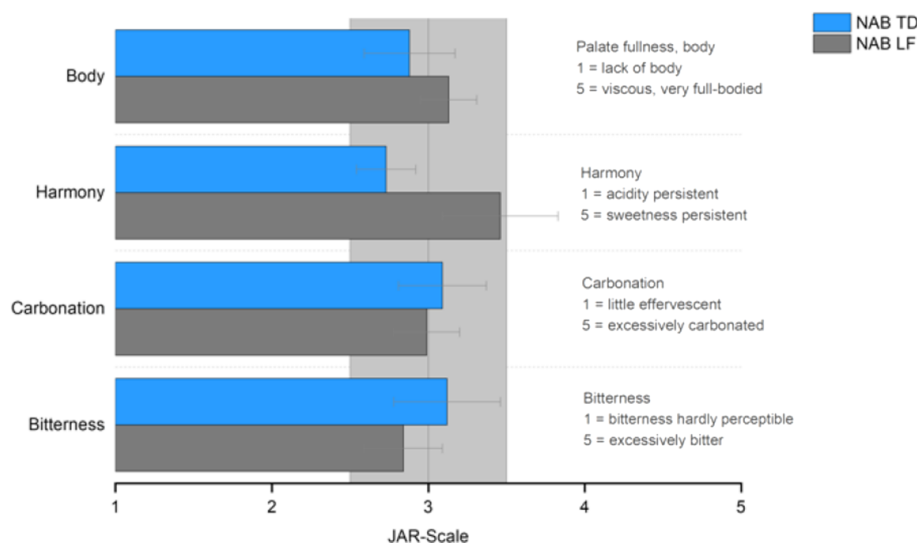


Figure 5. Additional attributes of NAB-TD and NAB-LF (mean \pm sd, $n = 14$) displayed on just-about-right scales with customised labelling. [Colour figure can be viewed at wileyonlinelibrary.com] [Colour figure can be viewed at wileyonlinelibrary.com]

($p < 0.001^{***}$) and 'caramel' ($p = 0.0127^*$). As expected, NAB LF produced by limited fermentation was rated significantly higher in this category than NAB TD. In particular, the attributes 'worty' ($\bar{x} = 3.65$) and 'sweet' ($\bar{x} = 3.91$) scored the highest values in NAB LF and 'honey' was characterised as strong ($\bar{x} = 3.02$). No off-flavours (class 3) were detected in either sample. The 'acidic' and 'phenolic' flavours in class 4 were rated higher in the thermally dealcoholised sample, NAB TD. Overall, the aroma profile of NAB TD was assessed as neutral, scoring medium intensities for the attributes 'worty' ($\bar{x} = 1.75$), 'acidic' ($\bar{x} = 1.75$; $p = 0.0007^{***}$) and 'phenolic' ($\bar{x} = 1.38$; $p = 0.6966$). The 'honey' ($\bar{x} = 1.25$; $p = 0.0663$) note was considered low. 'Bready', 'sweet', and 'caramel' flavours are not perceptible in this sample. Thermally dealcoholised beers are often used as the base for NAB blends; therefore, it is advantageous if they do not have any unpleasant aromas.

The results of the assessment of the non-volatile matrix (class 5-8) of the NABs are shown in Figure 5. In class 5, the palate fullness or body is rated on a 5 point scale, ranging from 1 = 'lack of body' to 5 = 'viscous, very full-bodied'. In this class, NAB LF ($\bar{x} = 3.13$) scored higher than NAB TD ($\bar{x} = 2.88$) but both can be considered to have a balanced body. Including class 6, 'harmony' in the evaluation scheme allows the differentiation between the NABs according to their sweet/sour profile. One end of the scale (1) represents a persistent sour final taste and the other end (5) is used for a distinctly sweet final taste. The middle (3) area represents a balanced sweet/sour ratio. The 'harmony' value of NAB TD is 2.73, which correlates well with the higher rating of the attribute 'acidic' (class 4) in the aroma profiling section. The 'harmony' in NAB LF was rated significantly higher ($\bar{x} = 3.46$; $p = 0.0008^{***}$). This value leans towards the sweet end of the scale and agrees with the rating in class 4. Class 7, 'carbonation' (NAB TD = 3.09; NAB LF = 2.99) and class 8, 'bitterness' (NAB TD = 3.12; NAB LF = 2.84) were both evaluated within the normal range for top fermented beers. No significant differences were found between both beers in these classes.

Top fermented NAB were chosen to show the potential for differentiation using the proposed scheme. However, this does not mean that the scheme should or can only be used for non-alcoholic, top fermented wheat beers. Additional attributes can also be used to better fit the beer style being assessed. Non-relevant terms for the beer style may also be removed. This provides flexibility and allows the tool to meet all the requirements of the beer style that needs to be evaluated.

Conclusions

The aim of this study was to determine the difference between alcoholic wheat beers and non-alcoholic wheat style beers from different production methods and to build a specific sensory scheme. In the first step, aroma active compounds in differently produced NABs were analysed instrumentally. A comparison of thermally dealcoholised beer and the original alcoholic wheat beer showed that aroma substances with low volatility remain throughout thermal dealcoholisation. However, the aroma active substances were detected in significantly lower concentrations and in different ratios (esters/fatty acids) than in alcoholic beers. Two manufacturing techniques for the production of NABs were compared in a second step. The aroma profile of non-alcoholic beers produced by limited fermentation was greatly influenced by wort carbonyls and hop treatment during production. Based on chemical data, it is possible to determine that different

production processes were used. NAB-TD is primarily characterised by a rose-like and phenolic aroma with NAB-LF by wort carbonyls.

Subsequent to aroma analysis, sensory analysis was performed. The collection of descriptors in the early steps of panel training resulted in a style specific lexicon, which panellists used to apply as attributes in the later validation of the scheme with NAB-TD and NAB-LF. The combination of descriptive and specific attributes for NABs (e.g. 'harmony') has the advantage of a holistic evaluation. Accordingly, the scheme can be considered as an 'extended scheme for NABs' (Figure 3), as it goes beyond a mere description of the aroma profile. Panellists have to be trained with a representative mix of NABs so that it can be used as a suitable tool. It can be applied and adopted for a new product group by a trained panel for beer and mixed beer beverages. However, the scheme in its present form is not a fixed. The choice and classification of attributes can be evaluated and adapted as necessary. An extension or reduction may be useful, depending on the panel and the questions that are asked of research or evaluation.

Author contributions

Magdalena Müller - conceptualisation, methodology, validation, formal analysis, investigation, writing (original draft), visualisation.
Martina Gastl - methodology, writing (review and editing), supervision.
Thomas Becker - supervision.

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Conflict of Interest

The authors declare no conflicts of interest.

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Supporting information

Additional supporting information may be found online in the Supporting Information section at the end of the article.