European Food Research and Technology (2023) 249:23–32
https://doi.org/10.1007/s00217-022-04050-7

ORIGINAL PAPER

Aroma profile of a gluten-free barley malt beer crafted to remove gluten using a barley malt extract with high peptidase activity

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Received: 1 March 2022 / Revised: 16 April 2022 / Accepted: 8 May 2022 / Published online: 1 June 2022

Abstract

Gluten-free beer (GFB) was produced from gluten-containing barley malt wort by adding an enzyme-enriched malt extract to the wort, intended for patients with celiac disease. Nevertheless, the aroma profile of such beer is unknown. The results indicated that the treatment reduced 3-methylbutyl acetate, 2-methylbutyl acetate, ethyl hexanoate, 2-methylpropyl acetate, and ethyl butyrate when compared with the reference. Moreover, foam, free amino nitrogen, aging stability, and color deviated significantly in the GFB. Although the values were below the odor threshold, analysis of aging components indicated increased amounts of heating, including 2-furfural and aging indicators, including phenylacetaldehyde. GC-O/MS following a descriptive profile test revealed that the attribute honey significantly increased. Further the shift in aroma composition of the GFB, long-chain free fatty acids (LCFFA) were analyzed, as they have been previously described to negatively correlate with ester metabolism and may be increased because of the technology applied. LCFFA analysis in wort revealed a significant increase in hexadecanoic and linoleic acids caused by the procedure. To clarify the findings, conventional barley malt worts were spiked with hexadecanoic acid, linoleic acid, and a mixture of these acids. A significant decrease in all esters concomitant with a honey-like aroma was observed when linoleic acid was added, but the aging analysis revealed no difference. Through adjustment of ester content to the reference, the spiked beers were less rated honey. The results indicate that aging components are sensed higher in the absence of esters; thus, by reducing LCFFA concentrations of gluten-free wort, the aroma of GFB could be enhanced.

Keywords Long-chain free fatty acids · Beer · Gluten-free · Celiac disease · Malt extract · Peptidase

Introduction

Several methods for producing gluten-free beers (GFBs) have been established. These are classified into two main strategies, namely, the application of gluten-free (GF) raw materials (I) or modified process engineering (II). Regarding raw materials, natural GF cereals, pseudocereals, or adjuncts are used; alternatively, non-GF cereals can be modified using classical and molecular breeding technologies. When using non-GF raw materials with process engineering, both the malting and brewing procedures must be intensified to degrade gluten, or exogenous enzymes have to be applied [1]. An alternative method is the use of a barley malt extract with high peptidase activity for the degradation of gluten in the wort. In this study, we produced GF wort using barley malt extract with high peptidase activity. The barley used for producing the enzyme-enriched extract underwent a modified malting procedure to achieve maximal enzymatic capacity and low initial gluten content [2], with the malting parameters 48% moisture, 18 °C temperature, and 8 days of germination yielding the best results. The hammer-milled barley malt underwent cold mashing at 4 °C for 15 min (ratio 1:2.5) to extract the gluten-specific peptidases. Then, the extract was concentrated using rotary evaporation at 50 °C until 40.4 brix was reached. Gluten-containing barley malt wort was incubated with malt extract in a ratio of 1:10 at 50 °C; we have previously described the determination of optimal parameters in this journal [3]. The resulting beer, especially the impact of this technological procedure on the

Contribution for the Special Issue: The chemistry behind malt and beer production – from raw material to product quality.

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GFB aroma, has not been described yet and is, therefore, the focus of the present study. Endogenous malt enzymes have the following advantages: (I) they are "optimized by nature" to hydrolyze proline- and glutamine-rich gluten proteins because the embryo needs amino acids and nitrogen during germination, (II) the technological process of cereal germination is well established (malting), (III) the enzymes are easy to extract, (IV) they originate from a cheap and naturally safe food source, (V) no genetic engineering is necessary [3], and (VI) the beers produced are in accordance to most national legislation. Whether the aroma composition of such processed GF barley malt beer is comparable with that of a conventional barley malt beer, which obtains gluten, remains uninvestigated. Overall, the aroma composition of barley malt-based beers is very complex and often characterized by synergistic or inhibitory effects. Although aroma active components are generally lower in lager beer fermented with *S. pastorianus* strains because of its matrix, slight changes in acetate ester composition result in a loss of masking effects, and consequently, an altered product [4]. Acetate esters, especially, are crucial for a pleasant beer aroma because of their low odor thresholds, such as isoamyl acetate (1.0 ppm, banana), ethyl acetate (15 ppm, solvent-like), isobutyl acetate (1 ppm, fruity), and phenylethyl acetate (0.5 ppm; rose). Moreover, medium-chain fatty acid ethyl esters, such as ethyl hexanoate (0.2 ppm, aniseed) and ethyl octanoate (0.1 ppm, green apple), were obtained from lager beer [5, 6]. There are several factors generally known to negatively suppress the ester formation, such as specific gravity, sugar profile, wort nitrogen content, oxygen concentration, temperature, yeast strain and growth factors, CO_2 and top pressure, pitching rate, and wort lipid content, especially fatty acids [7]. Because of the findings explained below, the focus of this research is laid on long-chain free fatty acids (LCFFA). LCFFAs are derived from cereal grains and, to a minor extent, hop [8]. During brewing, most fatty acids hexadecanoic acid (C16:0), octadecanoic acid (C18:0), oleic acid (C18:1), linoleic acid (C18:2), and linolenic acid (C18:3) are being retained in the spent grains and hot trub; nevertheless, 1–4% remain in the final wort [9]. Bravi et al. measured LCFFA concentrations in wort (12°P, 100% Pilsner malt) and obtained the following results: 0.425 mg/L tetradecanoic acid (C:14), 6.947 mg/L (C16:0), 0.426 mg/L palmitoleic acid (C16:1), 1.489 mg/L heptadecanoic acid (C17:0), 5.210 mg/L (C18:0), 0.248 mg/L (C18:1), and 0.673 mg/L (C18:2) [10]. Reportedly, the higher content of unsaturated fatty acids, particularly, may cause oxidative damage and stale flavor in the finished beer. The same authors described LCFFA in amber beer samples (80% Pilsner malt, 15% CaraRed, and 5% CaraMunich) as follows: 0.116 mg/L (C14:0), 0.981 mg/L (C16:0), 0.051 mg/L (C16:1), 0.882 mg/L (C18:0), 0.935 mg/L (C18:1), 2.465 mg/L (C18:2), and no information stated (C17:0) [11]. Although the values are not comparable because of differences in brewing procedures (temperature, lipolytic activity), malt composition, yeast metabolic activity, and beer maturation, they do indicate the concentration range of LCFFA in wort and beer.

This study aims to determine and optimize the quality attributes focusing on the aroma composition of a GFB crafted to remove gluten using a barley malt extract enriched with gluten-specific peptidases and to provide a profound explanation for the difference in aroma compared with a conventional barley malt beer focusing on the impact of LCFFA.

Materials and methods

Chemicals

The quality of all chemicals was an analytical grade or stated otherwise. Potassium hydroxide and ethanol were purchased from Merck (Darmstadt, Germany). Pentane, dichloromethane, trimethylsulfonium hydroxide, $NaSO_4$, hexadecanoic acid, and linoleic acid were purchased from Sigma Aldrich (Steinheim, Germany). Water was deionized using Thermo Scientific Barnstead MicroPure Water Purification System (Thermo Electron LED GmbH, Niederelbert, Germany).

Production of GFBs

Malt cv. Grace rich in gluten-specific peptidase activity was produced using a modified malting procedure according to Kerpes et al. [2]. The malting conditions were 48% moisture, 18 °C temperature, and 8 days of germination and yielded a peptidase activity of 27.2 U/kg malt. The optimized procedure increased the peptidase activity by a factor of 3 in comparison to raw barley. Previous findings by Knorr et al. [3], testing eight different cultivars from different locations, showed no significant impact (two-way ANOVA) on peptidase activity, which is why cv. Grace was chosen as it is well characterized for its brewing quality characteristics. Enzyme activity was analyzed as described previously by Kerpes et al. [2]. Following this, a malt extract was prepared from special malt and used in a 1:10 ratio for detoxification of a gluten-containing wort (10.6 P, 18 BE) using Weyermann wort concentrate type Bavarian Pilsner and Hallertauer Magnum according to [3]. The extract levels were adjusted. After pasteurization (1000 PU), the wort was fermented (10-L-Scale, 12 °C) using TUM 34/70 until extract was below 2°P. The beers were cooled down to 0 °C for 2 weeks, filtrated using coarse and fine kieselgur (ratio 1:2.3) as well as 1 g/L fine kieselgur as a regular dosage during filtration, and bottled at 0.65 g/L CO_2. All reference beers (REBs) were treated equally, except that no malt extract was added.

Production of spiked beers

Standardized wort (12°P, 18 BE) was produced by boiling commercial wort concentrate (Weyermann, type Bavarian Pilsner) with the addition of hop pellets (Hallertauer Magnum). Before fermentation, 56.08 µg hexadecanoic acid (H, C16:0), 36.47 µg linoleic acid (L, C18:2), or a mixture thereof (HL) were added to the wort. LCFFA was soluted in 10 mL ethanol before addition and trials were conducted in triplicate. To avoid the influence of the buffer, 10 mL ethanol was spiked to the reference wort before fermentation. Fermentation and filtration were conducted with all samples, as described in section "Production of the GFBs." For the final sensory evaluation, one part of the L and HL spiked beers were adjusted equal to the reference in terms of ester content using 3-methylbutyl acetate at concentrations of 0.77 mg/L for L and 0.7 mg/L for HL. The difference was calculated according to the analysis of the higher alcohols and esters (MEBAK 2.21.6).

LCFFA extraction

The samples were freeze-dried, and 3 g of the lyophilisate was extracted with 90 mL pentane–dichloromethane (2:1) and 15 g $NaSO_4$. Then, 1 mL of 0.00295% (m/v) pentadecanoic acid was added as internal standard. Extracts were filtered and 20 mL KOH was added, the emulsion was mixed in a separation funnel. Then, the aqueous phase was separated, adjusted to pH 2, and mixed with 90 mL pentane-dichloromethane (2:1) again. Na_2SO_4 was added to the organic phase and the suspension was filtered. The permeate was concentrated to 1 mL under vacuum, and 0.2 mL trimethylsulfonium hydroxide was added.

LCFFA analysis

The samples were analyzed using a Siemens SiChromat 3 gas chromatograph with FID connected to a Merck-Hitachi D2500 Integrator. The GC was equipped with a DB5 (J&W) 30 m × 0.25 mm capillary column, and helium was used as carrier gas at a flow rate of 1 mL/min (60 °C, split ratio 1:10). The temperature program was set at 100 °C for 5 min following heating to 250 °C at 5 °C/min stepwise.

Aroma analysis

Beer aroma component analysis was conducted via Headspace SPME GC-O/MS using a Siemens SiChromat II gas chromatograph (Siemens, Germany) coupled with a Finnigan MAT 8222 mass spectrometer (Finnigan, Germany). Beer samples (5 g) were sealed in a glass vial, tempered at 30 °C for 20 min, and analyzed. Incubation of the SPME fiber was set to 30 min. The fiber was injected and adsorbed aroma components were desorbed for 30 s at 250 °C.

Properties of the capillary separation column were ID 0.53 mm × 30 m, with a helium volume flow of 3 mL/min. The eluted fractions were split (1:1), and analysis using a sniffing system was conducted. Chromatograms were evaluated using NIST Library and MASPEC.

MEBAK beer quality attributes

The beer quality attributes shown in Table 1 were measured according to MEBAK [12].

Gluten quantitation in beer

Gluten was quantitated using a competitive R5 enzyme-linked immunosorbent assay (RIDASCREEN® Gliadin competitive No R7021, R-Biopharm, Darmstadt, Germany). Homogenized samples (1 mL) were mixed with extraction solution (9 mL, 60% v/v ethanol in water and containing 10% w/v fish gelatin) and analyzed according to the manufacturer's instructions. Samples were only analyzed at the recommended dilution of 1:500 for a better comparison of all experiments. This means that only gluten contents within the specified concentration range of the assay (10–270 mg gluten/kg) were identified. Absorption at 450 nm was measured using a Synergy™ H4 Hybrid Multi-Mode 96 Microplate Reader (Bio Tek, Winooski, USA), and data were evaluated using RIDA®SOFT Win software.

Table 1 List of MEBAK methods applied

Attribute	Method
Aging indicators	2.23.4
Alcohol	2.9.6.3
β-Glucan	2.5.2
BU	2.17.1
Color	2.12.2
Extract	2.9.6.3
FAN	2.6.4.1
Foam stability	2.18.2
Higher alcohols and esters	2.21.6
Original gravity	2.9.6.3
pH	–
Total nitrogen	2.6.1
TBI	2.4
Turbidity	2.14.1.2

Sensory analysis

A DLG-trained panel conducted DLG, descriptive profile test, and triangle analysis in a sensory room conforming to DIN 10962. The number of tasters was $N = 16$. Based on GC-O/MS results, the aroma attributes, i.e., berry, yeast, sweet, fruity, malt, and honey, were rated on a scale of 0 (not perceivable) to 5 (very intense) in full steps. For the LCFFA spiked beer, the attributes banana, fruity, malt, sweet, honey, berry, and oxidized were rated equally. Triangle analysis was conducted according to MEBAK sensory 3.1.3 and descriptive profile test according to 3.2.2 [13].

Statistical analysis

Data were statistically evaluated using the standard *t*-test in OriginPRO 2021 (OriginLab Corporation, USA), and differences were considered significant at $p < 0.05$.

Table 2 MEBAK quality attributes of the gluten-free beer (GFB) and the reference beers (REB)

Beer	Reference	Gluten-free
Alcohol (% m/v)	4.94 ± 0.05	4.87 ± 0.19
AAL (%)	88.05 ± 0.78	81.50 ± 0.90
β Glucane	207.00 ± 8.49	< 10 ± –
Bitter units (EBC)	18.0 ± 0.71	13.00 ± 0.00
Color (EBC)	7.35 ± 0.07	14.00 ± 1.00
FAN (mg/100 mL[a])	20.80 ± 1.84	42.00 ± 2.70
Foam (NIBEM)	319 ± 7.57	111 ± 4.51
Forcing test (*d*)	5 ± 0	3 ± 0
Original gravity (% m/m)	10.66 ± 0.01	11.33 ± 0.49
pH	4.47 ± 0.00	4.50 ± 0.40
TBI	22.00 ± 0.71	23.50 ± 0.00
Total nitrogen (mg/L[a])	71.60 ± 0.71	161.65 ± 2.95
Turbidity (25°)	1.67 ± 0.02	1.14 ± 0,00
Turbidity (90°)	2.40 ± 0.05	0.42 ± 0.00

[a]Based on 12% (w/w)

Results and discussion

Determination of the gluten content

The enzyme-linked immunosorbent assay (ELISA) results were consistent with previous research published by the authors [3] in this study, in which the untreated barley malt wort reached 246 ± 14 ppm. Then, gluten-specific peptidase malt extract was added to the barley malt wort and incubated at 50 °C for 24 h (for the detailed explanation, see introduction section). The gluten content of the wort treated with the malt extract high in peptidase activity decreased from 246 to < 10 ppm (limit of quantitation). The wort was incubated for 24 h without adding the malt extract. Here, the results were slightly higher at 275 ± 20 (not significant). As shown by Haas-Lauterbach et al., the variation is within the limits of the ELISA test utilized [14]. As no cross-contamination occurred during fermentation, the values of the final beer (< 10 ppm) did not differ significantly from the wort analysis. Hence, the wort and the resulting beer can legally be declared GF [15].

Characterization of the GFB

Standard quality attributes (MEBAK)

The procedure to remove gluten from barley malt wort using a malt extract with high peptidase activity extract had no significant impact on the alcohol content of the resulting GFB, and original gravity slightly increased. Thus, the AAL significantly decreased by 6.55% in the GFB (see Table 2), probably because of the ungelatinized amount of polysaccharides derived from (iodine test) the malt extract that cannot be metabolized by the yeast. The production of the malt extract is conducted below gelatinization temperature (64 °C) at 4 °C; thus, polysaccharides remain undegraded. The number of bitter units decreased because of the addition of the unhopped enzyme-enriched malt extract (dilution). Additionally, the malt extract is rich in proteins, observed here as total nitrogen, whereas iso-alpha acids are unspecifically adsorbed. The beer color showed a 90% increase in the GFB. This is due to not only the darker color of the malt extract (49 EBC) but also the thermal treatment. Moreover, pH and TBI were not significantly higher in the GFB despite the thermal treatment (50 °C, 24 h). TBI values of 22 and 23.5 are lower than the TBI measured in light-colored beers (28.8) by McGivney and Abishek [16]. Instead, turbidity levels (25°, 90°) increased early after 3 days of forcing. Higher turbidity levels may result from higher protein and LCFFA levels [17] (see section "LCFFA analysis"), which were both found to be increased in the GFB, as evidenced by the twofold increase in total nitrogen. Regarding foam stability, the REB obtained very good values of 319 (according to MEBAK), whereas the GFB decreased by 65%. This is probably due to the high degree of protein hydrolysis, as shown by the twofold increase in FAN levels. Regarding cytolytic attributes, *β*-glucan significantly decreased, which is possibly due to the remaining glucanase activity in the malt extract, which has its optimum between 40 and 45 °C (endo-*β*-1,4-glucanase) and up to 60 °C (endo-*β*-1,3-glucanase) [18].

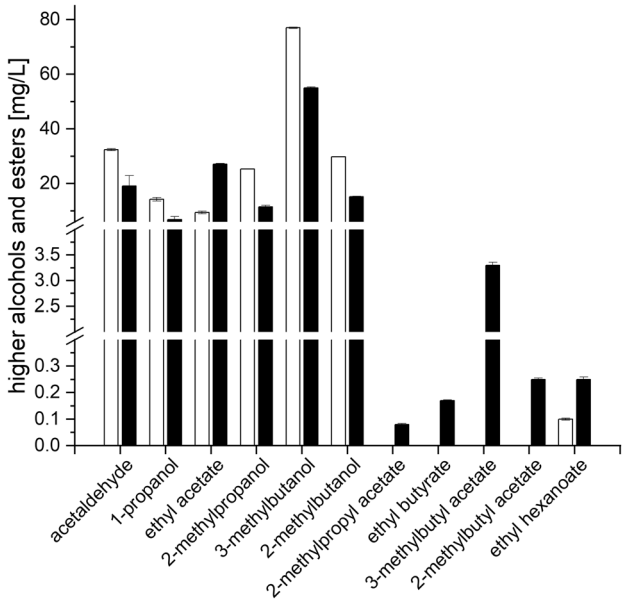

Fig. 1 Higher alcohols and esters of the GF beer (white bar) and the reference beer (black bar). Trials were conducted in triplicate; error bars are indicated

Analysis of the higher alcohols and esters

The analysis of the fermentation by-products revealed that all esters are strongly reduced (Fig. 1), with significant reductions occurring in ethyl acetate (− 65%), ethyl hexanoate (− 60%), 3-methylbutyl acetate, 2-methylbutyl acetate, 2-methylpropyl acetate, and ethyl butyrate (− 100%). By contrast, all alcohols significantly increased in the GFB between factors 1.4 (3-methylbutanol) and 2.2 (2-methylpropanol). According to Nordström [19], alcohols and acetyl-CoA are esterified to their corresponding acetate esters, which explains why alcohols were enhanced as educts, whereas esters were reduced, as ester metabolism by the yeast was inhibited because of LCFFA present in the gluten-free wort (GFW).

Determination of the aging indicators

The incubation of the wort with the malt extract occurred at a temperature of 50 °C [3] for maximal gluten-specific peptidase activity. This heat-dependent step influences especially the heat-dependent indicators, such as 2-furfural, 2-acetylfuran, 5-hydroxymethylfurfural, γ-nonalactone, diethyl succinate, and hexanal. The level of 2-furfural occurring via Maillard reaction increased by 93%, as did γ-nonalactone (99%) resulting from lipids [20].

Strecker aldehydes, 2-methylbutanal and 3-methylbutanal, increased moderately by 27% and 36%, respectively, in the GFB. The aldehydes benzaldehyde (154%) and phenylacetaldehyde (99%) resulting from Strecker degradation were higher in the GFB than in the REB. Although aging components significantly increased, all aroma components were below the odor threshold, as shown in Table 3. Generally, Strecker aldehydes indicate oxygen uptake during the brewing process [20]. Because the GFW and REW were treated equally, oxygen uptake must have occurred during the malt extract processing.

Table 3 Comparison of aging components measured in the gluten-free beer and the reference beer, as well as the threshold [4, 21]

Aging indicators[a]	Gluten-free beer (µg/L)	Reference beer (µg/L)	Threshold (µg/L)
3-Methylbutanal (o, a)	23.15 ± 0.13	16.95 ± 5.98	56[ba]
2-Methylbutanal (o, a)	8.87 ± 0.10	6.99 ± 2.58	45[b]
Hexanal	1.69 ± 0.11	1.92 ± 0.70	88[b]
2-Furfural (h, a)	14.63 ± 3.81	7.57 ± 0.92	15.15[b]
2-Acetylfuran (a)	3.30 ± 0.00	6.19 ± 0.21	513[b]
Benzaldehyde (o, a)	3.13 ± 0.42	1.23 ± 0.31	515[b]
5-Hydroxymethylfurfural (a)	n.d	n.d	35.78[ba]
2-Propionyl furan (a)	0.33 ± 0.03	0.43 ± 0.05	–
Phenyl acetaldehyde (o, a)	54.91 ± 1.47	27.59 ± 0.00	105[b]
Trans-2-nonenal	n.d	0.20 ± 0.15	0.11[b]
Diethyl succinate (a)	0.58 ± 0.11	0.62 ± 0.15	1200[c]
Ethyl nicotinate	1.72 ± 0.13	0.90 ± 0.04	4555[ba]
Ethyl phenylacetate (a)	1.32 ± 0.25	0.76 ± 0.10	–
γ-nonalactone (h, a)	15.00 ± 1.21	7.53 ± 0.03	607[ba]

[a]Mean values standard deviation of triplicate determinations

[b]Threshold acc. to Saison et al. [4]

[c]Threshold acc. to Vanderhaegen et al. [21]

[d]Odor threshold only

Besides, aging occurs through the esterification of organic precursor acids originating from hop or yeast to their corresponding ethyl esters. Here, diethyl succinate, ethyl nicotinate, and ethyl phenylacetate slightly increased, although because of their high odor threshold, their impact is of minor importance [21].

Nevertheless, except trans-2-nonenal, which was only discovered in the reference, none of the aging components revealed values that were close to the flavor or odor threshold [4, 21]. Thus, the treatment to produce GFB primarily increases heating indicators.

Sensory

The aroma of a GFB processed using malt extract with high peptidase activity remains unknown. Hence, both GFB and REB were analyzed using GC-O/MS to initially determine main odor aroma components (see Supplement S1). Here, the attributes berry, fruity, honey, malt, sweet, and yeast were determined to be the main flavor attributes. On the basis of the olfactory data, a descriptive profile test was designed, and the beers were evaluated in terms of their smell and taste by a trained panel ($N = 16$). The results are depicted in Fig. 2. Here, the attribute *honey* (REB = 0.99; GFB = 3.35) drastically increased, and attributes sweet (REB = 0.72; GFB = 1.7) and malt (REB = 1.25; GFB = 1.85) were noted by the panel. The same was stated of the taste, where honey was predominantly obtained. Additionally, all panelists correctly identified the GFB in a triangle test ($N = 16$, data not shown). Based on the literature, the aroma attribute honey is often found in long-term aged lager beer. The aroma impression was described by Kaneda et al. to result from phenylacetaldehyde, whereas others described the impression to occur because of a mixture of aroma active substances [20, 22]. This also matches with the findings of Jerkovic et al., discovering 600 aroma active substances in bee honey, whereas phenylacetaldehyde was one of the higher abundant substances obtained [23]. Despite phenylacetaldehyde being the most abundant aging substance in our findings, it is often associated with a rose-like flavor in beer. Together with increased concentration of 2-methylbutanal and 3-methylbutanal in GFB associated with a malty flavor and Maillard product 2-furfural often described as roasted and bready, the occurrence of honey-like flavor is plausible.

Thus far, it can be concluded that the use of a malt extract for gluten degradation significantly reduces all esters; especially, acetate esters, aging, and heating components are increased but all below the threshold, and the GFB aroma is described to obtain sweetish, malty, honey-like aroma.

To understand the shift in aroma composition of the GFB aroma impression, LCFFAs were analyzed as they were previously described to negatively correlate with ester metabolism [7]. Fatty acids are water soluble and nonheat resistant. Thus, because of the extraction procedure, where no heating step at high temperatures occurred, occurrence in higher concentrations is probable.

LCFFA analysis of the GFW and GFB

GFWs and GFBs were analyzed for LCFFA concentrations using a modified, self-developed method for determining C14:0, C16:0, C16:1, C17:0, C18:0, C18:1, and C18:2 in worts and beers. GFW analysis revealed that all seven LCFFA increased, with hexadecanoic acid (eightfold), oleic acid (52-fold), and linoleic acid (54-fold) being significantly higher (Fig. 3a). LCFFA concentrations in the REW showed no significant deviations when compared with the values obtained from the literature, except for myristic acid and oleic acid [10].

Nearly all fatty acids were reduced during fermentation, maturation, and storage in both the REB (exception: C16:1, not significant) and the GFB (Fig. 3b). Bravi et al. (2017) quantified LCFFA in amber beer samples (80% Pilsner malt, 15% CaraRed, and 5% CaraMunich) as follows: 0.116 mg/L (C14:0), 0.981 mg/L (C16:0), 0.051 mg/L (C16:1), 0.882 mg/L (C18:0), 0.935 mg/L (C18:1),

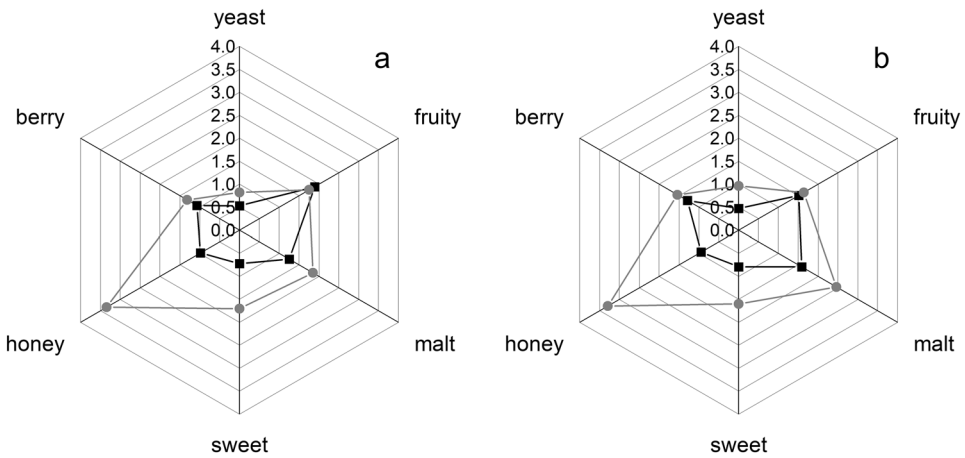

Fig. 2 Descriptive profile test of the gluten-free beer (gray) and the reference beer (black). On the left side **a** smell; right side **b** taste

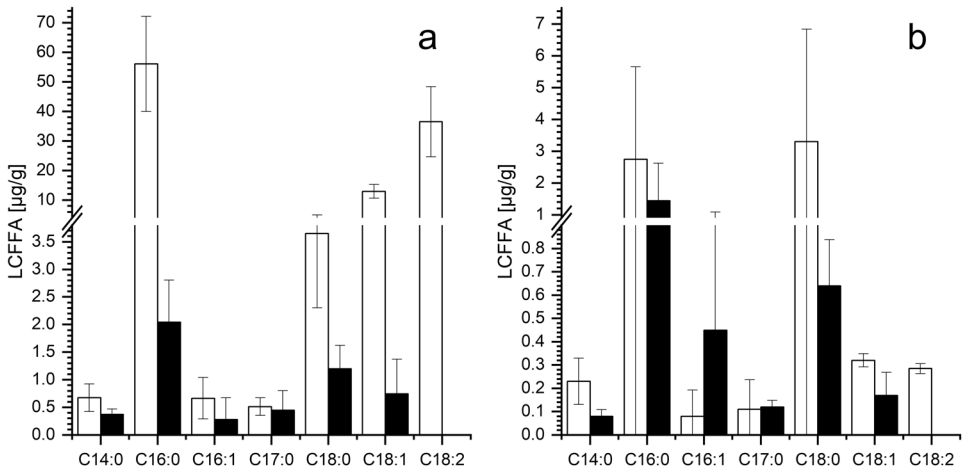

Fig. 3 Analysis of the saturated and unsaturated LCFFA in wort (**a**) and beer (**b**). White bars represent gluten-free samples, and black bars represent the reference. Trials were conducted in triplicate; error bars are indicated

2.465 mg/L (C18:2), no information provided (C17:0) [11]. Compared with these findings, the REB obtained lower values, however, with GFB, C14:0, C16:0, C16:1, and C18:0 were higher, whereas C18:1 and C18:2 were reduced. However, these values can only be considered as an indicator because there are no norm values available and other authors reported lower LCFFA levels [24]. The results clearly show that the use of the enzyme-enriched malt extract increased the total amount of LCFFA C:14–C:18:2 by 106 µg/g in the wort and 4.2 µg/g in the beer.

Imitation of the LCFFA impact on the GFB aroma using a spiked-model wort and beer

To determine if the increased amount of LCFFA in GFW is the lead influencing factor on the aroma of subsequent GFB, a model wort was spiked with 56 µg/g hexadecanoic acid (H), 36.5 µg/g linoleic acid (L), or a mixture thereof (HL). The concentrations were adjusted to the amounts measured in the GFW. The LCFFA analysis revealed moderate recovery rates of 51.2% for C16:0 and 32.1% for C18:2; therefore, both spiked fatty acids, although predissolved in ethanol, dissolved only partially. As there was no significant difference between H and HL for C16:0 or L and HL for C18:2 (see Supplement S2) and none of the other LCFFA showed a significant difference between the spiked beers and the reference, the variation of LCFFA in the model worts was found neglectable and therefore used for the production of the model beer.

Again higher alcohols and esters, beer quality attributes, and aging attributes were analyzed. Finally, the beers obtained were tasted by the panelists.

Characterization of the model beer

The analysis of the higher alcohols and esters revealed no significant difference between the model beers and the reference with acetaldehyde and propanol-1 (Fig. 4). As with the GFB, the model beer obtained 1.2–1.5 times higher values with 3-methylpropanol, 2-methylpropanol, and 2-methylbutanol when linoleic acid was spiked solely and in combination with hexadecanoic acid. By contrast, significant reduction was obtained from ethyl acetate (− 36% L and HL), 2-methylpropyl acetate (− 50% HL, n.d L), 3-methylbutyl acetate (− 64% L, − 58% HL), 2-methylbutyl acetate (− 38% L, − 53% HL), and ethyl hexanoate (− 56% L). Interestingly, the presence of saturated FA hexadecanoic

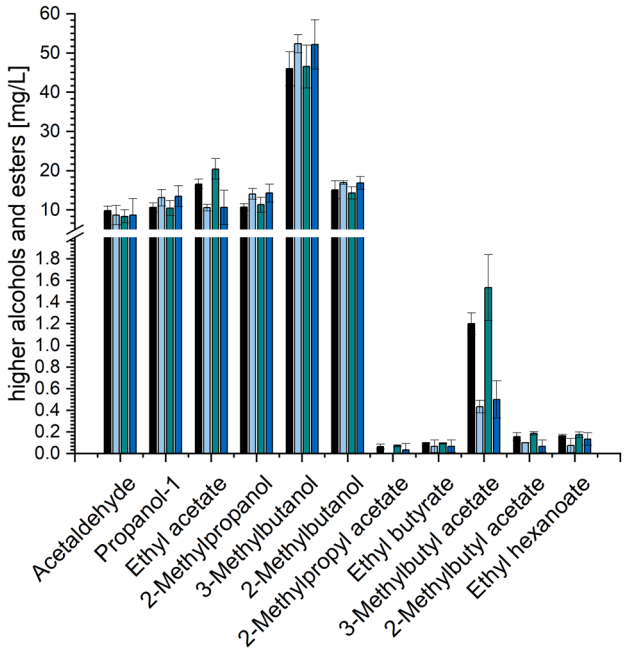

Fig. 4 Higher alcohols and esters of the spiked beers. Black columns represent reference; light blue columns, linoleic acid spiked beers; green columns hexadecanoic acid; and, dark blue columns linoleic acid + hexadecanoic acid. Trials were conducted in triplicate; error bars are indicated

acid increased nearly all esters, implying that saturated FA could serve as a tool to compensate for aroma losses in GFB. Focusing on linoleic acid, the data are consistent with previously obtained data from GFB; therefore, the deviation in ester concentration and composition of GFB can be attributed to unsaturated LCFFA.

A significant increase in aging indicators (see Supplement 3) in the model beers could only be observed with 3-methylbutanal (+26% L, +39% HL). When linoleic acid was added, 2-methylbutanal showed a slight increase (+4%) but decreased by 5% after the mixture (HL) was added. Both aldehydes are known to contribute to a malty, sweetish flavor, possibly causing honey-like flavor, and are usually produced via nonenzymatic, heat-induced Strecker degradation. In the brewing process, their formation is often caused by oxygen uptake during production. Neither phenylacetaldehyde (Strecker degradation) nor trans-2-nonenal (lipid oxygenation) showed significant deviation from the reference. All aroma components were lower compared with GFB, and all concentrations were below the threshold.

There were no significant differences in standard quality attributes (see Supplement 4). Regarding foam, LCFFA did not affect stability. Thus, the very low foam stability of the GFB could be due to proteinase A activity of yeast during GFW fermentation, as the altered extract composition (enzyme-rich malt extract) stresses the yeast or the difference in protein composition (Osborne fractionation) and molecular weight ratio [25, 26].

Sensory

Finally, the previously mentioned panelists evaluated the spiked beers in terms of smell (Fig. 5a) and taste (Fig. 5b). Again, the attribute honey was obtained, whereas L spiked

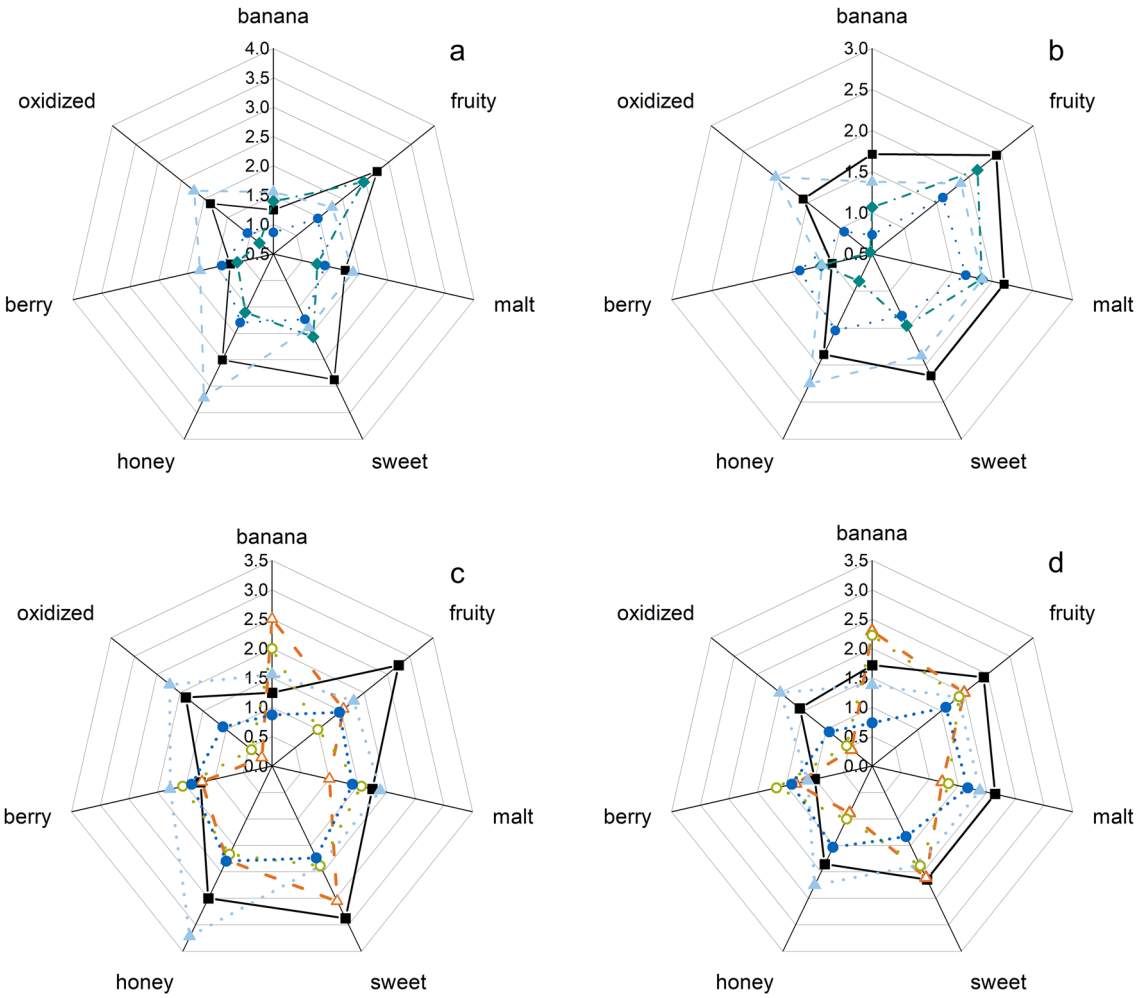

Fig. 5 Descriptive aroma analysis of the L, H, and HL spiked beers (**a** smell, **b** taste). Black squares represent the reference; green diamonds, the H spiked beers; light blue triangles, the L spiked beers; and dark blue circles, the HL spiked beers. Figure parts **c** (smell) and **d** (taste) show the aroma analysis of the spiked beers, where 3-methylbutyl acetate content was adjusted equal to the reference. Additionally, unfilled orange triangles represent the L + 3-methylbutyl acetate spiked beers, and unfilled light green circles, the HL + 3-methylbutyl acetate spiked beers

beer showed the highest rating in smell (3.2) and taste (2.3), followed by the reference. HL and H beers obtained lower values both in smell and taste. The H spiked beers (2.5) and the reference (2.8) were sensed as more fruity in smell. Moreover, the reference was sensed as the fruitiest in taste (2.4), followed by H (2.1), which is consistent with the findings from higher alcohols and esters, with the exception that H spiked beers were sensed lower in banana. The higher impression of sweet in the reference (2.8) cannot be attributed to the previous analysis, as this was also obtained in taste. L spiked beers were sensed higher regarding attribute oxidized in smell (2.2) and taste (2.0).

Because determining flavor thresholds (e.g., phenylacetaldehyde) in complex matrixes is difficult, an alternative approach was selected to prove the connection between low ester content and honey aroma, possibly caused by Strecker aldehydes being more dominant in esterless matrixes (GFB).

Therefore, the ester content of L and HL spiked beers were equally adjusted to the reference using 3-methylbutyl acetate. The sensory revealed a significant reduction of the aroma impression honey by 45% with L and 8% with HL (not significant) after the treatment in smell (Fig. 5c). Both beers were sensed lower regarding honey than the reference when ester content was equally adjusted. The treatment also affected the attribute oxidized, whereas impression was reduced by 60% (HL) to 90% (L). However, this trial should only be viewed as an indicator because this treatment did not fully imitate the native matrix, as observed with aroma banana, which increased by 61% in L and 150% in HL. Regarding taste (Fig. 5b), the same observation was made with honey (−61% L; −35% HL), oxidized (−78% L; −41% HL), and banana (+67% L; +200% HL).

Thus, a connection between unsaturated LCFFA and a honey-like impression is plausible.

Conclusion

This study indicated that a GFB produced using peptidase-enriched malt extract differed significantly from a gluten-containing REB. During GFW fermentation, the synthesis of all aroma active esters, such as ethyl acetate, 2-methylbutyl acetate, and 3-methylbutyl acetate, was inhibited. Additionally, GFB exhibited darker color and low foam stability, as well as lower aging stability (forcing test), although neither alcohol nor pH and turbidity were negatively affected. Regarding aging attributes, the procedure increases heat indicators, such as 2-furfural, γ-nonalactone, and Strecker aldehydes, including phenylacetaldehyde. The sensory impression of the GFB was honey, malt, and sweet.

The LCFFA analysis revealed that all fatty acids significantly increased in the GFB. The impact of LCFFA on GFB aroma was revealed by spiking experiments using linoleic acid and hexadecanoic acid (singular, mixture). In this study, unsaturated LCFFA specifically resulted in lower ester concentrations, whereas no aging indicators were affected; still, model beers were sensed honey in their aroma as well. By readjusting the ester content equal to the reference, the aroma impression was significantly reduced. Thus, the absence of aroma active esters in the GFB reduced the threshold of Strecker aldehydes, resulting in a honey-like flavor in the GFB. Saturated LCFFA seemed to have the opposite effect. In conclusion, to improve the aroma of the GFB presented, a selective technological re-movement of LCFFA or a more specific extraction procedure of the malt peptidases should be investigated.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s00217-022-04050-7.

Acknowledgements This research project was supported by the German Ministry of Economics and Technology (via AiF) and the FEI (Forschungskreis der Ernährungsindustrie e.V., Bonn). Project AiF 16971N.

Funding Open Access funding enabled and organized by Projekt DEAL.

Declarations

Conflict of interest The authors declare that they have no conflicts of interest.

Compliance with ethics requirements The authors declare compliance with ethics requirements.

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