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Influence of Malt Modifications on the Concentrations of Free Phenolic Acids in Wheat and Barley Malts

Process induced changes in free phenolic acids (caffeic-, cinnamic-, *p*-coumaric-, ferulic-, sinapic-, and vanillic acid) in wheat and barley grain, green malts, and kiln-dried malts were analysed by means of stable isotope dilution assays (SIDAs) and high performance liquid chromatography-tandem mass spectrometry (HPLC-MS/MS). Different steeping degrees, germination temperatures, and germination times were applied for malt production to study their influence on the concentrations of free phenolic acids. The most abundant free phenolic acid in wheat grain was ferulic acid (1.43 mg/kg dry mass) and vanillic acid (1.03 mg/kg dry mass) in barley grain. In wheat and barley green malts as well as in the corresponding kiln-dried malts, the amounts of some free phenolic acids increased in comparison to the grain, while others declined. Variation of the steeping degree, germination temperature, and germination time to manipulate the proteolytic and cytolytic degree of malt modification did not have a clear influence on the concentrations of free phenolic acids in kiln-dried wheat malts. However, in kiln-dried barley malts, a clear influence of the malt modifications on the concentrations of the acids was found. Especially the application of a higher steeping degree (48 %), germination temperature (18 °C), and germination time (7 days) yielded higher amounts of free phenolic acids in comparison to a lower steeping degree (42 %), germination temperature (12 °C), and germination time (5 days). Quantitation experiments revealed, for example, 1.54 mg of cinnamic acid and 4.09 mg of ferulic acid per kg dry mass of kiln-dried barley malt (highly modified malt) vs. 0.67 mg of cinnamic acid and 2.62 mg of ferulic acid per kg dry mass (undermodified malt), respectively.

Descriptors: Ferulic acid, grain, HPLC-MS/MS, malt, phenolic acids, stable isotope dilution analysis

1 Introduction

Free phenolic acids in wheat and barley malts are of special interest due to their antioxidative potential [1–4]. During beer brewing, these compounds can be extracted from malts in the mashing procedure and are, thus, also present in the wort, and, finally, in the beer. Mashing parameters like the mashing-in temperature and the pH value were shown to influence the concentration of free ferulic acid in the mash [5]. A mashing-in temperature of 40 °C and a pH of 5.8 were reported to be the best technological parameters for the enzymatic hydrolysis and solubilisation of bound ferulic acid. Furthermore, also parameters like the stirring regime, grist coarseness, and mash thickness were found to influence the concentrations of the acids [5].

Free phenolic acids are not only effective as antioxidants, and, thus, improve beer flavour stability [6, 7], they are also precursors for important aroma compounds. For example, decarboxylation of ferulic acid and *p*-coumaric acid leads to 2-methoxy-4-vinylphenol (4-vinylguaiacol) and 4-vinylphenol, respectively. This reaction is either thermally induced [8] or due to the enzymatic activity of the top-fermenting yeast *Saccharomyces cerevisiae* [9] used for fermentation. When present in concentrations above their respective odour thresholds, the clove-like aroma induced by these compounds is undesired in most beer types and is well-known as "phenolic off-flavour" [10]. But, in wheat beer, a Bavarian speciality beer brewed with at least 50 % of wheat malt, both are desired compounds responsible for the typical aroma of this beer type [11–17]. Thus, the amount of these free phenolic acids, and especially the control of their concentrations in malt, mash, and wort, is an important tool for the brewing industry [18, 19].

Recent studies reported on the occurrence of styrene in wheat beer [20], which is formed by the same pathway from another phenolic acid, namely cinnamic acid. Styrene is undesired in beer because of its toxicological relevance [21, 22] and is classified in group 2B (possibly carcinogenic to humans) by the International Agency for Research on Cancer (IARC) [23]. While some studies aimed at reducing the concentration of styrene in wheat beer by changing the fermentation regime [24, 25], another possibility might be the reduction of free cinnamic acid in the brewing process using appropriate malts.

Thus, the aim of the present study was (i) to produce differently modified malts applying different steeping degrees (42 %, 45 %,

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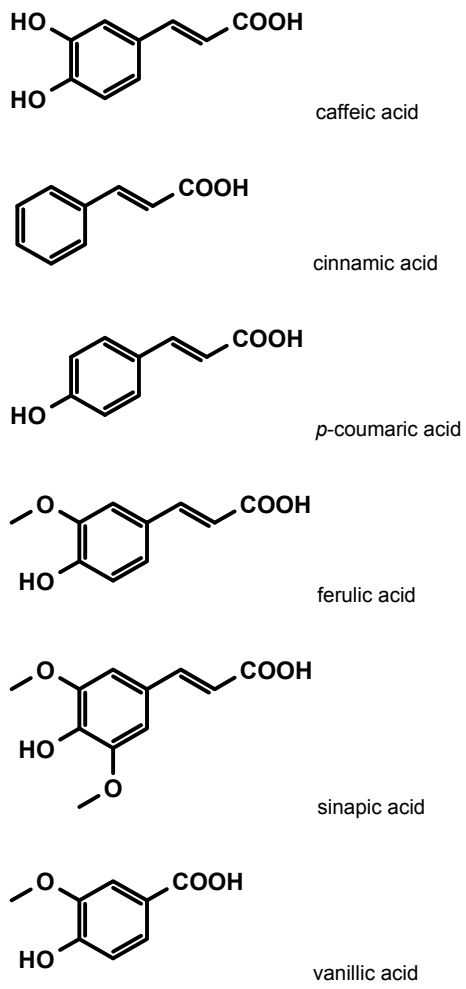

Fig. 1 Structures of the quantitated free phenolic acids

48 %), germination temperatures (12 °C, 15 °C, 18 °C), and germination times (5 days, 6 days, 7 days) and (ii) to investigate the influence of parameter variations on the concentrations of free phenolic acids (caffeic acid, cinnamic acid, *p*-coumaric acid, ferulic acid, sinapic acid, and vanillic acid; Fig. 1) in wheat and barley malts using a recently developed HPLC-MS/MS method on the basis of stable isotope dilution assays (SIDAs) [26].

Table 1 Steeping degree (SD), germination temperature (T), and germination time (t) as well as Kolbach index (as indicator for proteolytic modification) and viscosity (as indicator for cytolytic modification) of the analysed wheat and barley samples

Sample	SD (%)	T (°C)	t (days)	Kolbach index (%)	Viscosity (mPas related to 8.6 %)
wheat A	45	15	5	33.8	1.902
wheat B	45	15	7	38.2	1.887
wheat C	45	12	6	26.9	1,940
wheat D	45	18	6	41.2	1.743
wheat E	42	12	5	23.5	2.042
wheat F	48	18	7	46.1	1.782
barley A	45	15	5	40.0	1.487
barley B	45	15	7	44.1	1.420
barley C	45	12	6	40.9	1.497
barley D	45	18	6	36.2	1.437
barley E	42	12	5	35.2	1.787
barley F	48	18	7	41.7	1.419

2 Materials and methods

2.1 Grain and malt samples

All samples were produced in co-operation with the Chair of Brewing and Beverage Technology (Technical University of Munich; TUM School of Life Sciences Weihenstephan, Freising, Germany). Wheat samples were of the variety "Hermann", barley samples of the two row spring barley variety "Marthe" (with hull; both harvested in 2010, Swabian Alb, Germany).

Simultaneously, twenty-five "1 kg spelt batches" were malted using the "1 kg micro malting system" of the Chair of Brewing and Beverage Technology with 60 steeping facilities, climate chambers, and kilning system varying the steeping degree, germination temperature, and germination time using Response Surface Methodology [27]. A face-centered cube design with double replicated factorial and three times the center point was constructed using the software package Design-Expert® (Stat-Ease, Inc., Minneapolis, MN). Applying this design, malt samples were prepared to study the influence of steeping degrees, germination temperatures, and germination times by the following protocol: both wheat and barley grain were steeped until a steeping degree of 42 %, 45 %, or 48 % was obtained. Afterwards, samples were germinated at either 12 °C, 15 °C, or 18 °C for either 5 days, 6 days, or 7 days. Finally, the grain was standard kiln-dried at 50 °C for 16 h, at 60 °C for 1 h, at 70 °C for 1 h, and finally at 80 °C for 5 h. After kilning, rootlets and sprouts were removed and the malts were allowed to rest for 7 days prior to analysis. Having different possibilities of parameter combinations at hand, six samples of wheat and barley malts (centre point and extrema) were chosen independently from their brewing quality and were analysed in detail. The standard malt analyses were done according to MEBAK [28]. Table 1 specifies the differences in proteolytic and cytolytic indicators exemplified via the Kolbach index and viscosity related to the malting parameters. The concentrations of all phenolic acids (Fig. 1) were determined in the grain, in the green malts, and in the kiln-dried malts.

2.2 Chemicals

The following reference compounds and chemicals were obtained commercially: *p*-coumaric acid, sinapic acid, and vanillic acid (Fluka, Neu-Ulm, Germany), ferulic acid and cinnamic acid (Aldrich, Sigma-Aldrich-Chemie, Taufkirchen, Germany), acetonitrile, caffeic acid, ethyl acetate, formic acid, methanol, and sodium sulphate (Merck, Darmstadt, Germany). Ethyl acetate was of p.A. grade and was distilled on a Vigreux column before usage.

2.3 Stable isotopically labelled compounds

The following compounds were obtained commercially: [$^{13}C_3$]-caffeic acid, [$^{13}C_3$]-*p*-coumaric acid, [$^{2}H_3$]-ferulic acid, [$^{13}C_6$]-vanillin (Aldrich), and [$^{2}H_6$]-cinnamic acid (C/D/N Isotopes Inc., Québec, Canada). [$^{13}C_6$]-Vanillic acid was synthesised as recently described [26].

2.4 Extraction of free phenolic acids from grain, green malts, and kiln-dried malts and quantitation by stable isotope dilution assays (SIDAs)

The method used for extraction of free phenolic acids was recently established by our group [26]. Briefly, grain and malt samples were frozen with liquid nitrogen prior to grinding with an IKA M20 universal mill (Jahnke & Kunkel, Staufen im Breisgau, Germany). To the finely ground grain or malt powder (10 g each), methanol (200 mL) as well as the respective isotopically labelled internal standards [$^{13}C_3$]-caffeic acid, [$^{2}H_6$]-cinnamic acid, [$^{13}C_3$]-*p*-coumaric acid, [$^{2}H_3$]-ferulic acid, and [$^{13}C_6$]-vanillic acid were added. Sinapic acid was determined via [$^{2}H_3$]-ferulic acid. The amount of the respective standard was chosen close to the concentration of the analyte, determined in a preliminary experiment. Then, the sample was stirred for 200 min at room temperature, centrifuged (5 min, 4000 rpm, 15 °C; Multifuge X3 FR; Thermo Scientific, Schwerte, Germany), and filtered. The solvent was removed by means of a rotary evaporator (35 °C, 20 mbar) and the residue was dissolved in water (20 mL) and, finally, ultrasonified for 2 min. Next, the solution was extracted with ethyl acetate (4 x 20 mL), the combined extracts were dried over anhydrous sodium sulphate, filtered, and finally evaporated again to dryness. Prior to HPLC-MS/MS analysis, the residue was dissolved in water/acetonitrile (1 mL; 9/1, v/v) by ultrasonification and the sample was membrane filtered (Whatman Spartan® 13, 0.45 µm; GE Healthcare, Freiburg, Germany).

2.5 High performance liquid chromatography-tandem mass spectrometry (HPLC-MS/MS)

HPLC-MS/MS was performed by means of a triple-quadrupole tandem mass spectrometer (TSQ Quantum Discovery; Thermo Electron, Dreieich, Germany) coupled to a Surveyor high-performance liquid chromatography system (Thermo Finnigan, Egelsbach, Germany) equipped with a thermostated (20 °C) autosampler. Separation was performed using a Synergi 4u Fusion RP column (150 mm x 2 mm I.D.) connected to a C18 pre-column (4 mm x 2 mm I.D.; both Phenomenex, Aschaffenburg, Germany) and a mobile phase consisting of an aqueous formic acid solution (0.1 %; A) and a formic acid solution in acetonitrile (0.1 %; B). The flow rate was 0.2 mL/min with an elution gradient of 90 % A and 10 % B at the beginning, raised to 65 % B and 35 % A during 30 min, and finally to 100 % B within 2 min and kept for 3 min. Precursor ions were generated using positive electrospray ionisation (ESI^+) with a spray voltage of 3500 V, sheath gas pressure of 35 arb, auxiliary gas of 5 arb, and a capillary temperature of 320 °C. The mass spectrometer was operated in the single reaction monitoring (SRM) mode. The most intensive product ion for each compound was used as quantifier and the second intensive one was used as qualifier (Table 2).

Response factors (R_f; Table 2) were calculated by analysing mixtures of known amounts of the unlabelled analyte and the corresponding labelled standard in five different ratios (5:1, 3:1, 1:1, 1:3, 1:5) showing a good linearity for all analytes (R^2 = 0.99; except for sinapic acid, for which R^2 = 0.98) in the applied range.

2.6 Determination of recoveries, limits of detection (LoDs), and limits of quantitation (LoQs)

The recoveries as well as limits of detection and quantitation for all analytes were determined as recently described [26]. Thereby, the recoveries ranged between 93.1 % and 102.9 %, the LoDs between 0.01 mg/kg dry mass and 0.15 mg/kg dry mass, and the LoQs between 0.05 mg/kg dry mass and 0.25 mg/kg dry mass.

2.7 Determination of dry mass of grain, green malts, and kiln-dried malts

The finely ground grain or malt powder (10 g each) was added to sea sand (25 g) in an evaporating dish and mixed thoroughly. The samples were dried in a drying oven at 102 °C for 5 h. After cooling down to room temperature in an exsiccator, dry mass was determined by weight difference of the samples before and after drying.

3 Results

3.1 Concentrations of free phenolic acids in the grain

For wheat grain, the most abundant phenolic acid was ferulic acid (1.43 mg/kg dry mass) followed by vanillic acid (1.26 mg/kg dry mass) and *p*-coumaric acid (0.23 mg/kg dry mass). The concentrations of caffeic acid and cinnamic acid were below the LoQ, the concentration of sinapic acid was below the LoD (Table 3A).

In contrast, in barley grain, vanillic acid showed the highest concentration (1.03 mg/kg dry mass), followed by ferulic acid (0.59 mg/kg dry mass), caffeic acid (0.42 mg/kg dry mass), and *p*-coumaric acid (0.28 mg/kg dry mass). The concentrations of cinnamic acid and sinapic acid were below the LoD (Table 3B).

Using this wheat and barley grain, green and kiln-dried malts were prepared by applying different steeping degrees, germination temperatures, and germination times. It was already known

Table 2 Selected ions (*m/z*) of analytes and stable isotopically labelled standards as well as response factors (R_f) used in stable isotope dilution assays

compound	analyte		standard			R_f
	precursor ion (*m/z*)	product ions (*m/z*)[a]	isotope labelling	precursor ion (*m/z*)	product ions (*m/z*)[a]	
caffeic acid	181	145, **163**	$^{13}C_3$	184	148, **166**	0.88
cinnamic acid	149	103, **131**	$^{2}H_6$	155	108, **136**	0.99
p-coumaric acid	165	119, **147**	$^{13}C_3$	168	121, **150**	0.85
ferulic acid	195	145, **177**	$^{2}H_3$	198	145, **180**	0.87
sinapic acid[b]	225	175, **207**	$^{2}H_3$[b]	198	145, **180**	0.69
vanillic acid	169	**93**, 151	$^{13}C_6$	175	**99**, 157	1.00

[a] Product ions in bold were used for quantitation;
[b] Quantitated via [$^{2}H_3$]-ferulic acid

that an increase of the germination temperature or time intensifies the cytolytic modification of the malt, and that higher degrees of steeping lead to a better proteolytic modification [29]. Independent from the resulting quality specifications, the experimental design was used to create different degrees of proteolytic and cytolytic modifications caused by variation of the malting parameters and to study their influences on the concentrations of free phenolic acids.

3.2 Influence of germination times on the concentrations of free phenolic acids in green and kiln-dried malts

First, the steeping degree (SD) and germination temperature (T) were kept constant and the influence of the germination time (t) on the concentrations of free phenolic acids from the wheat and barley grain via the corresponding green malts to the kiln-dried malts was investigated. A germination time of either 5 or 7 days was chosen (Table 1; wheat A, wheat B, barley A, and barley B).

In wheat A (SD 45 %, T 15 °C, t 5 days; Table 3A), *p*-coumaric acid (0.83 mg/kg dry mass) and ferulic acid (2.35 mg/kg dry mass) showed the highest concentrations in the kiln-dried malt, whereas the highest amount of sinapic acid was found in the green malt (4.08 mg/kg dry mass). The concentration of vanillic acid decreased constantly from the grain via the green malt to the kiln-dried malt (0.45 mg/kg dry mass). The concentration of caffeic acid was below the LoQ in the grain and green malt and below the LoD in the kiln-dried malt, while cinnamic acid could only be quantitated in the kiln-dried malt (0.45 mg/kg dry mass). In wheat B (SD 45 %, T 15 °C, t 7 days; Table 3A) comparable trends were observed for all phenolic acids.

In barley A (SD 45 %, T 15 °C, t 5 days; Table 3B), the highest amounts for cinnamic acid, *p*-coumaric acid, ferulic acid, and vanillic acid were measured in the green malt, while the concentrations decreased in kiln-dried malt. In contrast, sinapic acid was highest in the kiln-dried malt (1.57 mg of /kg dry mass) and caffeic acid could only be quantitated in the grain. In barley B (SD 45 %, T 15 °C, t 7 days; Table 3B), the concentration trends of all phenolic acids were similar compared to barley A, except for ferulic acid, which showed its highest concentration in the kiln-dried malt (3.50 mg/kg dry mass) and not in the green malt.

A comparison of the kiln-dried wheat and barley malts produced with the same malting parameters showed a clear tendency to higher concentrations of free phenolic acids in the barley malts (cf. Table 3A and Table 3B).

Thus, a variation of the germination time only showed a minor influence on the release of bound phenolic acids, e.g., barley B with a prolonged germination resulted in a slight increase of the free phenolic acids in the respective kiln-dried malts.

Table 3A Concentrations of free phenolic acids in wheat grain and the corresponding green and kiln-dried malts: wheat A (SD 45 %, T 15 °C, t 5 days), wheat B (SD 45 %, T 15 °C, t 7 days)

analyte	conc. (mg/kg dry mass)[a]				
	grain	wheat A		wheat B	
		green malt	kiln-dried malt	green malt	kiln-dried malt
caffeic acid	< LoQ[b]	< LoQ[b]	< LoD[d]	< LoD[d]	< LoD[d]
cinnamic acid	< LoQ[c]	< LoD[e]	0.45 (± 0.02)	< LoD[e]	0.48 (± 0.03)
p-coumaric acid	0.23 (± 0.01)	0.82 (± 0.02)	0.83 (± 0.02)	0.53 (± 0.03)	0.65 (± 0.03)
ferulic acid	1.43 (± 0.02)	2.04 (± 0.06)	2.35 (± 0.18)	1.49 (± 0.10)	2.68 (± 0.06)
sinapic acid	< LoD[d]	4.08 (± 0.83)	1.86 (± 0.25)	3.70 (± 0.12)	1.73 (± 0.30)
vanillic acid	1.26 (± 0.07)	0.71 (± 0.04)	0.45 (± 0.02)	0.84 (± 0.10)	0.41 (± 0.03)

[a] Mean values of triplicates. Relative standard deviations (RSDs) in parentheses; [b] LoQ = 0.25 mg/kg dry mass; [c] LoQ = 0.05 mg/kg dry mass; [d] LoD = 0.15 mg/kg dry mass; [e] LoD = 0.03 mg/kg dry mass

Table 3B Concentrations of free phenolic acids in barley grain and the corresponding green and kiln-dried malts: barley A (SD 45 %, T 15 °C, t 5 days), barley B (SD 45 %, T 15 °C, t 7 days)

analyte	conc. (mg/kg dry mass)[a]				
	grain	barley A		barley B	
		green malt	kiln-dried malt	green malt	kiln-dried malt
caffeic acid	0.42 (± 0.01)	< LoD[c]	< LoD[c]	< LoD[c]	< LoD[c]
cinnamic acid	< LoD[b]	0.95 (± 0.04)	0.85 (± 0.13)	1.64 (± 0.21)	1.19 (± 0.06)
p-coumaric acid	0.28 (± 0.01)	1.46 (± 0.17)	0.84 (± 0.06)	1.36 (± 0.07)	1.07 (± 0.06)
ferulic acid	0.59 (± 0.03)	3.16 (± 0.03)	2.94 (± 0.07)	2.95 (± 0.17)	3.50 (± 0.21)
sinapic acid	< LoD[c]	1.10 (± 0.06)	1.57 (± 0.03)	1.16 (± 0.04)	2.41 (± 0.10)
vanillic acid	1.03 (± 0.07)	1.67 (± 0.10)	0.97 (± 0.05)	2.08 (± 0.02)	1.06 (± 0.06)

[a] Mean values of triplicates. Relative standard deviations (RSDs) in parentheses; [b] LoD = 0.03 mg/kg dry mass; [c] LoD = 0.15 mg/kg dry mass

3.3 Influence of germination temperatures on the concentrations of free phenolic acids in green and kiln-dried malts

For the next series of experiments, SD (45 %) and t (6 days) were kept constant, while germination temperatures of either 12 °C or 18 °C were applied for malt production (Table 1; wheat C, wheat D, barley C, and barley D).

In wheat C (SD 45 %, T 12 °C, t 6 days; Table 4A), caffeic acid was not detectable in both malts. Concentrations of cinnamic acid (0.61 mg/kg dry mass) and *p*-coumaric acid (0.84 mg/kg dry mass) increased from the grain via green malt to kiln-dried malt. In wheat D (SD 45 %, T 18 °C, t 6 days; Table 4A), similar trends were found compared to wheat C, except for *p*-coumaric acid showing the highest amount in the green malt (0.76 mg/kg dry mass).

In barley C (SD 45 %, T 12 °C, t 6 days; Table 4B), the concentration of caffeic acid was also below the LoD in both malts. In contrast, the amounts of cinnamic acid, *p*-

coumaric acid, and vanillic acid clearly increased from the grain to the green malt, but declined in the kiln-dried malt. Ferulic acid and sinapic acid showed maximum concentrations in the kiln-dried malt (3.24 mg/kg dry mass and 1.64 mg/kg dry mass, respectively). The concentration trends in barley D (SD 45 %, T 18 °C, t 6 days; Table 4B) were similar to barley C, except for ferulic acid showing its highest concentration in the green malt (3.87 mg/kg dry mass).

Comparing the samples with the same malting parameters, the concentrations of all free phenolic acids were higher in the kiln-dried barley malts compared to wheat malts, which was also shown for the malts produced with varying germination times (samples A and B; Table 3A and Table 3B). This fact was most pronounced for vanillic acid and cinnamic acid, which showed a nearly 3-fold and 1.9-fold higher amount (cf. Table 4A and Table 4B).

Thus, similar to variations in germination time, also different germination temperatures did not have a clear influence on the concentrations of free phenolic acids in kiln-dried wheat and barley malts.

3.4 Influence of a simultaneous variation of steeping degree, germination temperature, and germination time on the concentrations of free phenolic acids in green and kiln-dried malts

For the simultaneous approach, minimum and maximum values of the parameters steeping degree, germination temperature, and germination time were applied for malt preparation (Table 1; wheat E, wheat F, barley E, and barley F).

In wheat E (SD 42 %, T 12 °C, t 5 days; Table 5A), caffeic acid as well as cinnamic acid were not detectable in the green malt and only cinnamic acid was present in kiln-dried malt (0.49 mg/kg dry mass). Both *p*-coumaric acid and ferulic acid showed their highest concentrations in the kiln-dried malt (0.99 mg/kg dry mass and 2.22 mg/kg dry mass, respectively). In wheat F (SD 48 %, T 18 °C, t 7 days; Table 5A), the amounts of most free phenolic acids in the kiln-dried malt were higher compared to wheat E, except for vanillic acid and *p*-coumaric acid.

Thus, in kiln-dried wheat malts, a simultaneous variation of all malting parameters did not show a clear influence on the concentrations of free phenolic acids. Nevertheless, cinnamic acid, ferulic acid, and sinapic acid were present in higher concentrations in the kiln-dried malt produced with higher parameters.

In barley E (SD 42 %, T 12 °C, t 5 days, Table 5B), the amount of caffeic acid was below the LoD in both malts. Cinnamic acid (0.78 mg/kg dry mass), *p*-coumaric acid (0.96 mg/kg dry mass) as well as vanillic acid (1.20 mg/kg dry mass) showed their highest concentrations in the green malt and their concentrations declined in the kiln-dried malt, whereas the amount of ferulic acid was almost equal in both malts. In barley F (SD 48 %, T 18 °C, t 7 days; Table 5B), the trends were comparable to barley E, except for ferulic acid showing the highest amount in the kiln-dried malt. In general, higher concentrations for all free phenolic acids were analysed in the kiln-dried malt of barley F (except caffeic acid: both below the LoD).

However, in contrast to the wheat samples, a simultaneous increase of all malting parameters clearly influenced the concentrations of free phenolic acids in kiln-dried barley malts, which were all higher in the kiln-dried malt of barley F produced with the maximum values of all malting parameters compared to barley E produced with the minimum values (cf. Table 5A and Table 5B). Additionally, kiln-dried malts of barley E and barley F showed the greatest differences in concentrations of free phenolic acids of all samples (Table 5B).

4 Discussion

The results suggested that especially barley malt shows potential for reduced concentrations of free phenolic acids by varying

Table 4A Concentrations of free phenolic acids in wheat grain and the corresponding green and kiln-dried malts: wheat C (SD 45 %, T 12 °C, t 6 days), wheat D (SD 45 %, T 18 °C, t 6 days)

analyte	conc. (mg/kg dry mass)[a]				
	grain	wheat C		wheat D	
		green malt	kiln-dried malt	green malt	kiln-dried malt
caffeic acid	< LoQ[b]	< LoD[d]	< LoD[d]	< LoD[d]	< LoQ[b]
cinnamic acid	< LoQ[c]	0.08 (± 0.03)	0.61 (± 0.02)	0.41 (± 0.09)	0.54 (± 0.01)
p-coumaric acid	0.23 (± 0.01)	0.57 (± 0.03)	0.84 (± 0.00)	0.76 (± 0.02)	0.69 (± 0.02)
ferulic acid	1.43 (± 0.02)	1.26 (± 0.04)	2.54 (± 0.16)	1.66 (± 0.04)	2.41 (± 0.13)
sinapic acid	< LoD[d]	2.48 (± 0.24)	1.35 (± 0.15)	5.05 (± 0.44)	2.17 (± 0.07)
vanillic acid	1.26 (± 0.07)	0.45 (± 0.07)	0.37 (± 0.02)	1.10 (± 0.22)	0.37 (± 0.03)

[a] Mean values of triplicates. Relative standard deviations (RSDs) in parentheses; [b] LoQ = 0.25 mg/kg dry mass; [c] LoQ = 0.05 mg/kg dry mass; [d] LoD = 0.15 mg/kg dry mass

Table 4B Concentrations of free phenolic acids in barley grain and the corresponding green and kiln-dried malts: barley C (SD 45 %, T 12 °C, t 6 days), barley D (SD 45 %, T 18 °C, t 6 days)

analyte	conc. (mg/kg dry mass)[a]				
	grain	barley C		barley D	
		green malt	kiln-dried malt	green malt	kiln-dried malt
caffeic acid	0.42 (± 0.01)	< LoD[c]	< LoD[c]	< LoD[c]	< LoD[c]
cinnamic acid	< LoD[b]	2.43 (± 0.19)	1.17 (± 0.11)	1.73 (± 0.15)	1.04 (± 0.11)
p-coumaric acid	0.28 (± 0.01)	1.57 (± 0.05)	1.17 (± 0.00)	1.66 (± 0.13)	1.15 (± 0.07
ferulic acid	0.59 (± 0.03)	3.11 (± 0.15)	3.24 (± 0.24)	3.87 (± 0.16)	3.29 (± 0.24)
sinapic acid	< LoD[c]	0.72 (± 0.04)	1.64 (± 0.07)	1.00 (± 0.01)	2.32 (± 0.12)
vanillic acid	1.03 (± 0.07)	1.64 (± 0.14)	1.03 (± 0.08)	2.64 (± 0.26)	1.08 (± 0.12)

[a] Mean values of triplicates. Relative standard deviations (RSDs) in parentheses; [b] LoD = 0.03 mg/kg dry mass; [c] LoD = 0.15 mg/kg dry mass

the malting parameters steeping degree, germination temperature, and germination time. This offers mitigation strategies for styrene in wheat beer by a reduced input of its precursor free cinnamic acid into the brewing process. Especially application of the lowest malting parameters steeping degree 42 %, germination temperature 12 °C, and germination time 5 days (sample E) resulted in > 50 % reduced concentration of free cinnamic acid in the kiln-dried barley malt compared to sample F (Table 5B). Wheat malts were influenced to a lesser extent showing a reduction of only about 30 % of cinnamic acid between wheat E and wheat F (Table 5A).

However, application of lower parameters for malt production did not only result in lower concentrations of free cinnamic acid, but also in reduced concentrations of free *p*-coumaric acid (in barley malt) and ferulic acid (in both malts). Due to the fact that these compounds are precursors for important aroma-active compounds in wheat beer, high concentrations in the mash, and, thus, in the kiln-dried malts are desired. Fortunately, a tendency towards a higher reduction of cinnamic acid compared to *p*-coumaric acid and ferulic acid was achieved when lowering all malting parameters. To the best of our knowledge, this is the first report about the influence of the malting parameters steeping degree, germination time, and germination temperature on the concentrations of several free phenolic acids in wheat and barley malts.

Nevertheless, variation of malting parameters is a limited tool, because, on the one hand, the concentration of free cinnamic acid in the kiln-dried malt cannot be reduced selectively. On the other hand, some malts did not show satisfying cytolytic and proteolytic activities resulting in suboptimal brewing properties. Therefore, malts of wheat and barley cultivars with low concentrations of free cinnamic acid and high concentrations of free pcoumaric acid and ferulic acid should be favoured. Differences in the total concentration of ferulic acid were already analysed by *Zupfer* et al. [30], depending on the barley cultivar. Furthermore, *Moore* et al. [31] demonstrated that not only the genotype plays an important role for the concentration of phenolic acids in bran samples, but also the environment of growth including factors like temperature stress and solar radiation using 20 hard winter wheat varieties grown during the 2001 season at two locations. *Coghe* et al. [32] showed that the concentration of free ferulic acid can be quite different in congress wort brewed with malts of different wheat cultivars, e.g., 1.83 mg/L in wort brewed with Legat compared to 2.85 mg/L in wort brewed with Genghis. Similar results were obtained by *Vanbeneden* et al. [33], who investigated congress worts brewed with nine different barley varieties. The analysed amounts of free ferulic acid and *p*-coumaric acid ranged between 0.95–3.45 mg/kg dry mass and 0.53–1.20 mg/kg dry mass, respectively, depending on the malted barley variety used for mashing. *Yang* et al. [34] showed that the total amounts of ferulic acid and vanillic acid (after acidic hydrolysis) in wheat changed remarkably during a germination time of 9 days, but an increase in both concentrations was not measured before the fifth day of germination. In addition, the authors reported on a slight influence of the steeping time (24 h or 48 h).

Up to now, most studies investigated the influence of the kilning regime (e.g., the kilning temperature and kilning time) on the concentrations of free phenolic acids in malts [35–39]. For example, *Samaras* et al. [37] analysed the amounts of free phenolic acids in barley, in the corresponding green malt, in stewed malt, in pale malt, in lager malt, and in roasted malts (e.g., cara malt, crystal malt, black malt, chocolate malt, and roasted malt). Thereby, decreasing concentrations of free ferulic acid, *p*-coumaric acid, and vanillic acid from the grain to the green malt were found, which is not in accordance with our study. Further, they analysed the highest concentrations of these free phenolic acids in the kiln-dried malts (pale malt and lager malt). In our study, the concentrations mostly decreased from the green malt to the kiln-dried malt, with ferulic acid being the only compound show-

Table 5A Concentrations of free phenolic acids in wheat grain and the corresponding green and kiln-dried malts: wheat E (SD 42 %, T 12 °C, t 5 days), wheat F (SD 48 %, T 18 °C, t 7 days)

analyte	conc. (mg/kg dry mass)[a]				
	grain	wheat E		wheat F	
		green malt	kiln-dried malt	green malt	kiln-dried malt
caffeic acid	< LoQ[b]	< LoD[d]	< LoD[d]	< LoQ[b]	< LoQ[b]
cinnamic acid	< LoQ[c]	< LoD[e]	0.49 (± 0.01)	0.08 (± 0.01)	0.68 (± 0.03)
p-coumaric acid	0.23 (± 0.01)	0.82 (± 0.07)	0.99 (± 0.07)	0.71 (± 0.03)	0.87 (± 0.04)
ferulic acid	1.43 (± 0.02)	1.20 (± 0.03)	2.22 (± 0.08)	1.65 (± 0.16)	2.57 (± 0.01)
sinapic acid	< LoD[d]	1.20 (± 0.31)	1.02 (± 0.17)	3.89 (± 0.38)	2.12 (± 0.23)
vanillic acid	1.26 (± 0.07)	0.24 (± 0.04)	0.38 (± 0.02)	1.08 (± 0.17)	0.38 (± 0.02)

[a] Mean values of triplicates. Relative standard deviations (RSDs) in parentheses; [b] LoQ = 0.25 mg/kg dry mass; [c] LoQ = 0.05 mg/kg dry mass; [d] LoD = 0.15 mg/kg dry mass; [e] LoD = 0.03 mg/kg dry mass

Table 5B Concentrations of free phenolic acids in barley grain and the corresponding green and kiln-dried malts: barley E (SD 42 %, T 12 °C, t 5 days), barley F (SD 48 %, T 18 °C, t 7 days)

analyte	conc. (mg/kg dry mass)[a]				
	grain	barley E		barley F	
		green malt	kiln-dried malt	green malt	kiln-dried malt
caffeic acid	0.42 (± 0.01)	< LoD[c]	< LoD[c]	< LoD[c]	< LoD[c]
cinnamic acid	< LoD[b]	0.78 (± 0.05)	0.67 (± 0.04)	2.65 (± 0.01)	1.54 (± 0.14)
p-coumaric acid	0.28 (± 0.01)	0.96 (± 0.10)	0.73 (± 0.03)	1.53 (± 0.09)	1.47 (± 0.07)
ferulic acid	0.59 (± 0.03)	2.65 (± 0.18)	2.62 (± 0.18)	2.60 (± 0.03)	4.09 (± 0.17)
sinapic acid	< LoD[c]	0.56 (± 0.04)	1.09 (± 0.03)	1.42 (± 0.10)	3.35 (± 0.13)
vanillic acid	1.03 (± 0.07)	1.20 (± 0.08)	0.96 (± 0.01)	2.47 (± 0.15)	1.26 (± 0.09)

[a] Mean values of triplicates. Relative standard deviations (RSDs) in parentheses; [b] LoD = 0.03 mg/kg dry mass; [c] LoD = 0.15 mg/kg dry mass

ing increasing concentrations in barley B, barley C, and barley F. The maximum kilning temperature in our study (80 °C) was comparable to the maximum temperature used in their study (85 °C and 87 °C, respectively) for the production of pale and lager malt. Thus, the differences in concentration trends cannot be explained by temperature effects. Further, according to Samaras et al., the concentrations of free ferulic acid, *p*-coumaric acid, and vanillic acid rapidly decreased in black, chocolate, and roasted malts, which is probably due to the high temperatures (220 °C–229 °C) applied. To prove the decarboxylation of phenolic acids at elevated temperatures, Samaras et al. exemplarily analysed 2-methoxy-4-vinylphenol (4-vinylguaiacol) in black, chocolate, and roasted malt, revealing concentrations between 267 µg/kg dry mass and 439 µg/kg dry mass.

Further, *Woffenden* et al. [36] reported about the effect of the kilning time and moisture content of barley malt on the concentrations of some phenolic acids. An increase of free ferulic acid after 27 h of kiln-drying in two different malts (one produced with a standard kilning procedure and another produced with a rapid kilning procedure) was analysed, accompanied with a decreasing moisture content.

Inns et al. [38] also compared two different kilning procedures, a standard regime (SKR) and a rapid regime (RKR). The concentrations of free ferulic acid, *p*-coumaric acid, and vanillic acid changed with rising temperatures in the analysed barley malts. While the amount of ferulic acid increased until a temperature of 80 °C (in SKR malt) and 70 °C (in RKR malt), respectively, higher temperatures (up to 120 °C) resulted in a decrease. For *p*-coumaric acid, the same trend was observed for both malts at temperatures ≤ 70 °C, while its concentration decreased at temperatures between 70 °C and 85 °C. A further increase in temperature did not influence the amount. Similarly, vanillic acid showed the highest concentration at a temperature of 70 °C (in RKR malt) and 80 °C (in SKR malt), respectively.

These changes in the concentrations of free phenolic acids during kilning are probably linked to enzymatic activity: as phenolic acids are bound to arabinoxylans in grain [40] and enzymes are responsible for their release [41], it is very likely that in the early stage of kilning the involved enzymes find their temperature optima [39] and, therefore, the concentrations of free phenolic acids clearly increased during this period. At higher temperatures (> 80 °C), however, degradation of free phenolic acids may already begin. Another possible explanation for higher levels of free phenolic acids in malts produced at temperatures up to 80 °C might be changes in the matrix, followed by a better extractability of these compounds, as discussed by *Maillard* and *Berset* [35].

5 Conclusion

Malt modification, caused by varying steeping degrees, germination times, and germination temperatures, showed a clear influence on the concentrations of free phenolic acids in this study. Thus, a mitigation strategy for the undesired styrene in wheat beer can be provided by reducing the input of its precursor free cinnamic acid into the brewing process using malts produced with appropriate malting parameters. However, high amounts of the desired aroma precursors free *p*-coumaric acid and ferulic acid must be maintained to get a wheat beer with the typical clove-like aroma impression, which is well-known and claimed by consumers. Further investigations are currently under way to prove the assumption that the use of malts with a reduced concentration of free cinnamic acid for brewing really results in lower concentrations of this phenolic acid in the mash and wort, and, finally, of its decarboxylation product styrene in the beer. Additionally, the production of beers with an intensified clove-like, smoky aroma impression using malts with elevated amounts of free *p*-coumaric acid and ferulic acid or of beers with improved antioxidative properties due to the use of malts with an increased free phenolic content might also be of general interest.

Although reducing the amount of free cinnamic acid in malts is a good starting point for styrene mitigation, reactions during the brewing process may also play a role in the release of this precursor. It is already known that the majority of phenolic acids are insolubly bound to arabinoxylans in barley grain [40] and the enzymatic release during malt preparation is comparatively low. But due to enzyme activity, they are present in high amounts as soluble bound esters in the mash. There, the release of free phenolic acids, e.g., by feruloyl esterase still being active after the kilning process [42], occurs during the mashing process [33]. The extent of this release during mashing, the possible decarboxylation of free phenolic acids during brewing, and the influence of the initial concentrations of free phenolic acids in malts on beers will be the topic of future studies.

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