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Guaiacol and 4-Methylphenol as Specific Markers of Torrefied Malts. Fate of Volatile Phenols in Special Beers through Aging

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ABSTRACT: Phenol-specific extracts of 12 Belgian special beers were analyzed by gas chromatography hyphenated to olfactometry (AEDA procedure) and mass spectrometry (single ion monitoring mode). As guaiacol and 4-methylphenol were revealed to be more concentrated in brown beers (>3.5 and >1.1 μg/L, respectively), they are proposed as specific markers of the utilization of dark malts. Analysis of five differently colored malts (5, 50, 500, 900, and 1500 °EBC) allowed confirmation of high levels of guaiacol (>180 μg/L; values given in wort, for 100% specialty malt) and 4-methylphenol (>7 μg/L) for chocolate and black malts only (versus respectively <3 μg/L and undetected in all other worts). Monitoring of beer aging highlighted major differences between phenols. Guaiacol and 4-methylphenol appeared even more concentrated in dark beers after 14 months of aging, reaching levels not far from their sensory thresholds. 4-Vinylphenols and 4-ethylphenols, on the contrary, proved to be gradually degraded in POF(+)-yeast-derived beers. Vanillin exhibited an interesting pattern: in beers initially containing <25 μg/L, the vanillin concentration increased over a 14 month aging period to levels exceeding its sensory threshold (up to 160 μg/L). Beers initially showing an above-threshold level of vanillin displayed a decrease during aging.

KEYWORDS: *special malts, phenols, brown beers, beer aging*

INTRODUCTION

Brewers have long been particularly interested in phenolic compounds, especially for their recognized antioxidant potential but also for their contribution to beer aroma.[1,2] Hydroxybenzoic and hydroxycinnamic acids are both found in their free and arabinoxylan-esterified forms in the cereal cell wall.[3−5] Aromatically inactive in beer because of their high threshold values, these compounds can be thermally decarboxylated to volatile phenols during malting, roasting, wort boiling, wort clarification, and pasteurization, thus contributing to the overall flavor.[6,7] Furthermore, the phenylacrylic decarboxylase activity of POF(+) top-fermenting yeasts (Pad1 phenotype) and of contaminating microorganisms such as Enterobacteriaceae spp., lactic and acetic bacteria, and *Brettanomyces/Dekkera* spp. can cause additional enzymatic decarboxylation during fermentation.[8−10] Issued from ferulic and *p*-coumaric acid decarboxylation,[11,12] 4-vinylguaiacol and 4-vinylphenol (respective thresholds of 125 and 170 μg/L) are well-known contributors of a clove-like[11,13] flavor to Belgian white beers[14] (barley malt and unmalted wheat used as ingredients) and to German wheat beers[15,16](made with malted wheat).

In the presence of *Brettanomyces* (used for lambic and gueuze beers and for one Belgian Trappist beer[17]), vinylphenols can be reduced further to ethylphenols, mainly 4-ethylguaiacol and 4-ethylphenol (horse-like; respective thresholds of 130 and 150 μg/L).[13,18]

Amber and brown beers have a long-established tradition in Belgium. Caramel and torrefied malts give them a particular color and flavor derived from nonenzymatic browning reactions occurring during kilning. Caramel malts are issued from green malts, or rewetted kilned malts, kept at a temperature below 60−75 °C until the content of the grain is liquefied.[19] Maillard reactions can further take place during kilning or in the torrefactor drum, where the temperature is then increased to 120−180 °C according to the desired color. For elaboration of torrefied malts (chocolate and black malts) with intense coffee aromas, Pilsen malts or chit malts are heated in a roasting cylinder from about 75 to 175 °C and then slowly to 215 °C.[20] With increasing color, various well-known odorant Strecker aldehydes, pyrazines, and furans are synthesized and/or degraded according to the temperature/water activity program applied.[21−23] Decarboxylation of free phenolic acids and lignin degradation have been described to produce additional roasted, chocolate, and coffee flavors.[24]

The aim of the present work was first to identify specific phenolic markers of torrefied malts in dark beers. Phenols were further quantified in a large series of specialty malts and in special beers through aging.

EXPERIMENTAL PROCEDURES

Beer Samples. Twelve commercial Belgian special beers were investigated (Table 1). Among these, seven were provided by breweries, just after bottling or refermentation (BL1−BL4, AM1, BR1, and TR1). All were bottle-refermented except AM1, which was filtered, and BL3, which was centrifuged. For the Trappist TR1, *Brettanomyces* yeast strains were also used for secondary and third fermentations. Two additional Belgian amber beers (AM2 and AM3, for which caramel malts were used), two dark brown beers (BR2 and BR3, for which torrefied malts were used), and a white Belgian beer (W1) were bought at a local supermarket.

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Table 1. Characteristics of Belgian Special Beer Samples

type	beer	alcohol (%)	original extract (g extract/100 g wort)	characteristics
blond	BL1	8.5	16.3	Pilsen malt, unfiltered, bottle refermentation
	BL2	7.5	14.9	Pilsen malt, organic beer (organic version of BL1), unfiltered, bottle refermentation
	BL3	6.3	14.4	Pilsen malt,[a] POF(+) yeast, centrifuged, flash pasteurization
	BL4	7.5	16.8	Pilsen malt,[a] POF(+) yeast, unfiltered, bottle refermentation, flash pasteurization
dry-hopped Trappist	TR1	6.2	13.3	caramel malt,[a] POF(+) yeast, *Brettanomyces* for secondary fermentation and bottle refermentation, dry hopping, unfiltered
amber	AM1	12.0	20.8	caramel malt, filtered
	AM2	4.5	10.93	caramel malt,[a] POF(+) yeast, filtered
	AM3	5.4	12.77	caramel malt, filtered
brown	BR1	8.0	19.8	torrefied malt (7%),[a] POF(+) yeast, light filtration, bottle refermentation
	BR2	6.5	15.58	torrefied malt,[a] POF(+) yeast
	BR3	5.2	12.71	torrefied malt (12%), bottle refermentation
white Belgian	W1	4.9	11.8	Pilsen malt and wheat, spices (orange peels, coriander),[a] POF(+) yeast, centrifuged

[a]The POF(+) yeast descriptor is given only for yeasts that have been previously investigated in our laboratory for the PAD1 phenotype.[32]

Malt Samples. Five commercial barley malts (*Hordeum vulgare* L.) were provided by the Malterie du Château (Beloeil, Belgium): Pilsen malt (5 EBC), caramel malts (Cara 50 °EBC and coffee 500 °EBC; final kilning temperature = 125 and 180 °C, respectively), and torrefied malts (chocolate 900 °EBC and black 1500 °EBC; final roasting temperature = 225 °C).

Chemicals. Methanol (99.9%) and dichloromethane (99.9%, redistilled twice prior to use) were purchased from Romil (Prosan, Belgium). Ultrapure water (Milli-Q water purification system, Millipore, Bedford, MA, USA) was used. 2-Methoxy-4-vinylphenol (4-vinylguaiacol, 98%), dodecane (external standard for vanillin glucoside extraction, 99.9%), decane (external standard for phenol extraction, 99.9%), naphthol (internal standard for phenol extraction, 99.5%), guaiacol (98%), 4-methylguaiacol (97%), 4-ethylguaiacol (98%), 4-vinylphenol (solution 10% in propylene glycol), 4-ethylphenol (98%), 4-hydroxy-3-methoxy-α-methylbenzyl alcohol (apocynol, 97%), diethyl ether (99,9%), octyl-β-D-glucopyranoside (internal standard for vanillin glucoside extraction, >98%), and β-glucosidase from almond were obtained from Sigma-Aldrich (Bornem, Belgium). *p*-Cresol (4-methylphenol, 99%) came from Acros Organics (Geel, Belgium). Vanillin (99%), sodium sulfate (99%), sodium chloride (99.5%), chloroform (99%), and potassium hydroxide (85%) were from Merck (Darmstadt, Germany). Hydrochloric acid (37%) was purchased from Fisher Scientific (Leicestershire, UK). Amberlite XAD-2 resin (Supelco, Bellefonte, PA, USA) (with a pore size of 9 nm and a specific area of 330 m^2/g) was sequentially washed with methanol and diethyl ether (each for 4 h) in a Soxhlet and stored in methanol at 4 °C.

Wort Production. Laboratory congress worts were produced in an LB 8 electronic normal-version mashing unit (Lochner, Germany) according to European Brewery Convention procedures (EBC Analytica 1998, method 4.5.1). Fifty grams of finely ground malt was added to 200 mL of water at 46 °C. The temperature was kept at 45 °C under constant stirring for 30 min. The caramel malts (Cara 50 and coffee 500) and torrefied malts (chocolate 900 and black 1500) were used with Pilsen malt at the ratio of 50:50 w/w (quantitations given for 100% specialty malt). The temperature was raised to 70 °C at 1 °C/min, then 100 mL of water was added, and the temperature was kept for 1 h at 70 °C with constant stirring before the mixture was cooled to room temperature and the weight to 450 g with water. After paper filtration (MN 614 × 32 cm diameter; Macherey-Nagel GmbH), the wort samples were stored at −32 °C until extraction.

Samples Aging. All beers were stored in a dark room at 20 °C before analysis.

Phenol-Specific Liquid−Liquid Extraction Procedure. All extractions were carried out in duplicate, according to a procedure derived from Callemien.[25] First, 50 mL of beer, 100 μL of IST (50 mg/L naphthol; 100 μg/L in beer), 1 mL of 37% hydrochloric acid, and 6.45 g of sodium chloride were mixed. After complete salt dissolution, 150 mL of chloroform/methanol (3:1, v/v) was added and the mixture stirred for 10 min at 1500 rpm. The lower organic phase was retained, whereas the aqueous phase was extracted a second time in the same manner. The collected 300 mL of organic phase was then shaken with 50 mL of 10% potassium hydroxide solution for 10 min at 1500 rpm. The upper aqueous phase (pH 13) was recovered and the lower organic phase extracted once again. The pH of the aqueous phase was then adjusted to pH 9.0 with hydrochloric acid and extracted twice with 25 mL of dichloromethane after stirring for 10 min at 1500 rpm. The combined organic phase was dried with anhydrous sodium sulfate, and 0.1 mL of EST (50 mg/L decane) was added to the extract before concentration to 0.5 mL in a Danish-Kuderna apparatus at 45 °C (total concentration factor = 100). The final extracts were stored at −81 °C and analyzed by GC-FID, GC-MS (Figure 1), and GC-O.

Vanillin Glucoside Enzymatic Extraction Procedure. First, 150 mL of BR1, 500 μL of IST (1 g/L octyl-glucopyranoside; 3.3 mg/L in beer), and 6 g of Amberlite XAD-2 resin thoroughly rinsed with Milli-Q water (approximately 400 mL) were poured into a 250 mL Schott flask. Samples were shaken in a dark room for 2 h and then poured into a glass column for separation. Aqueous eluate from the XAD-2 resin was drained, and 50 mL of water and 25 mL of diethyl ether were consecutively poured for washing. Glucosides were finally eluted with 25 mL of methanol. The extract was evaporated to dryness and resuspended in 25 mL of acetate buffer (pH 5). Enzymatically treated samples and controls were incubated at 35 °C for 2 h, with or without β-glucosidase (14 mg). Both were extracted three times with 15 mL of diethyl ether (10 min, 1000 rpm). The combined organic phase was dried with anhydrous sodium sulfate, and 0.5 mL of EST (20 mg/L dodecane) was added to the extract before concentration to 0.5 mL in a Danish-Kuderna apparatus at 39 °C. The final extracts were stored at −81 °C and semiquantitatively analyzed by GC-MS (just using the area ratio to the IST and the IST concentration; relative recovery factors and molar response coefficients fixed at 1).

Gas Chromatography Hyphenated to Mass Spectrometry (GC-MS). Electronic impact (EI) mass spectra were recorded at 70 eV on a ThermoFinnigan Trace MS mass spectrometer connected to a ThermoFinnigan Trace GC 2000 gas chromatograph equipped with a splitless injector (250 °C; opened 0.8 min after injection of 1 μL) and an apolar CP-Sil 5 CB MS capillary column (50 m × 0.32 mm i.d., 1.2

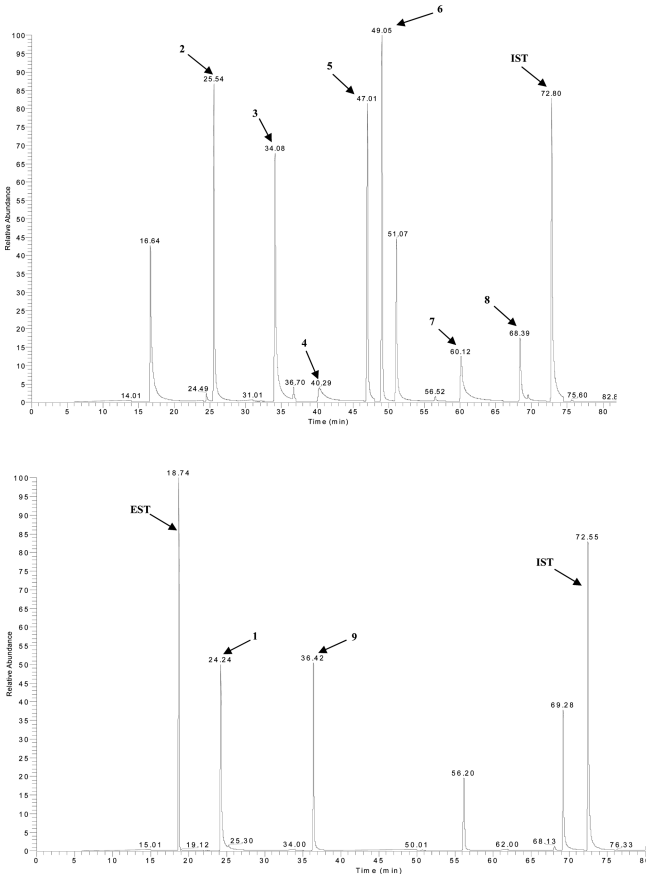


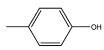
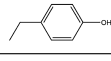
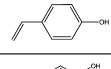
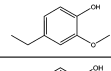
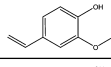
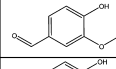
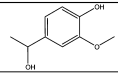
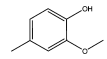
Figure 1. GC-MS-SIM chromatograms of our nine phenols spiked (20 mg/L) into a beer extract.

μm film thickness). The carrier gas was helium, and the pressure was set at 100 kPa. The oven temperature was raised from 36 to 85 °C at 20 °C/min, then to 145 °C at 1 °C/min, and finally to 250 °C at 3 °C/min. For all compounds, the single ion monitoring (SIM) mode was applied (selected m/z given in Table 2). As depicted in Figure 1, two separate SIM programs were used to analyze our nine phenols. Xcalibur software was used for spectral recording throughout the chromatographic separation. The mass spectra obtained were compared to those of commercial standards and to the NIST library.[26] Quantification of phenols was done by standard addition to beer or to wort.

Gas Chromatography−Olfactometry (GC-O). Beer extracts (1 μL) were analyzed on a Chrompack CP9001 gas chromatograph equipped with a splitless injector maintained at 250 °C and opened 0.5 min postinjection. The carrier gas was nitrogen at a flow rate of 1 mL/min. Compounds were analyzed with a wall-coated open tubular (WCOT) apolar CP-Sil 5 CB capillary column (50 m × 0.32 mm i.d., 1.2 μm film thickness). The oven temperature was programmed to rise from 36 to 85 °C at 20 °C/min, then to 145 °C at 1 °C/min, and finally to 250 °C at 3 °C/min. This temperature was maintained for 30 min. To assess the olfactory potential of the extract, the column was connected to a GC-O port (Chrompack) maintained at 250 °C. The effluent was diluted with a large volume of air (20 mL/min) prehumidified with an aqueous copper(II) sulfate solution. The extracts of three beers (BL1, TR1, and BR1) were analyzed by aroma extract dilution analysis (AEDA)[27] to estimate the relative impact of each odorant. According to the AEDA method, the extracts were diluted stepwise with dichloromethane (1 + 2 by volume). The flavor dilution (FD) factor is defined as the highest dilution at which the compound can still be detected (FD = 3^n, where n + 1 is the number of dilutions applied to the extract until there was no detection by GC-O).

Statistical Analyses. Analyses were carried out in duplicate, and Student−Newman−Keuls tests were performed with SAS software version 9.2 (SAS Institute, Inc., Cary, NC, USA) to compare the means. Values sharing no common letter are significantly different (p < 0.05).

Table 2. Phenolic Concentrations (μg/L) in Fresh Belgian Special Beers Determined by GC-MS[a]

N°	RI CP-Sil5	Substance	Structure	Odor (GCO)	Threshold in beer (µg/l)	Selected m/z	BL1	BL2	BL3	BL4	TR1	AM1	AM2	AM3	BR1	BR2	BR3	W1
1	1046	4-Methylphenol		Burned, dentist	20	108/107	0.3^{c} (1)	0.3^{c}	0.2^{c}	0.4^{c}	0.5^{c} (1)	0.6^{c}	0.25^{c}	0.2^{c}	1.6^{b} (9)	1.1^{b}	3.8^{a}	ud^{c}
2	1063	Guaiacol		Roasted, coffee	70	124/109	1.1^{d} (27)	1.3^{d}	0.8^{d}	1.5^{d}	0.87^{d} (9)	0.6^{d}	0.9^{d}	0.4^{d}	7.62^{b} (27)	3.5^{c}	9.7^{a}	1.2^{d}
3	1140	4-Ethylphenol		Cresol	150	122/107	2.2^{b} (9)	0.8^{b}	0.7^{b}	2.8^{b}	1601^{a} (27)	2.5^{b}	ud^{b}	ud^{b}	6.6^{b} (27)	ud^{b}	ud^{b}	0.3^{b}
4	1191	4-Vinylphenol		Phenolic	170	120/105	470.1^{b,c} (3)	485.1^{b,c}	0.2^{d}	30.9^{d}	0.2^{d} (0)	16.4^{d}	330.9^{b,c}	8.2^{d}	166.7^{d} (3)	570.3^{a}	173.9^{c,d}	504.8^{a,b}
5	1257	4-Ethylguaiacol		Clove	130	152/137	0.7^{b} (1)	0.6^{b}	0.3^{b}	0.7^{b}	1603^{a} (243)	0.5^{b}	1.6^{b}	0.2^{b}	12.6^{b} (9)	0.7^{b}	23.9^{b}	0.1^{b}
6	1289	4-Vinylguaiacol		Clove	125	150/135	1197.4^{b} (729)	1724.6^{a}	35^{e}	115.6^{e}	0.5^{e} (3)	52.2^{e}	375.8^{c,d,e}	18.7^{e}	374.4^{c,d} (243)	700.6^{b,c}	205.9^{d,e}	603.2^{b,c,d}
7	1365	Vanillin		Vanilla	50	152/151	66.1^{b} (9)	113.8^{a}	6.7^{b}	9^{b}	6.9^{b} (3)	4.5^{b}	27.4^{b}	2.3^{b}	25.9^{b} (9)	38.1^{b}	21.7^{b}	19.6^{b}
8	1451	Apocynol		Vanilla	50	153/93	1.5^{a} (3)	2.9^{a}	2.2^{a}	2^{a}	2^{a} (1)	1.6^{a}	0.5^{a}	0.4^{a}	1.7^{a} (1)	3.3^{a}	2.4^{a}	1.4^{a}
9	1180	4-Methylguaiacol		Burnt	20	-	undetected											

[a]Assays in duplicate. GC-O AEDA analyses of phenol-specific extracts of three beers (BL1, TR1, and BR1) (FD in parentheses = 3^n with n + 1 = number of dilutions of the extract before no detection − precision: n ±1 or a factor 3 between FD values).

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RESULTS AND DISCUSSION

Volatile Phenols in Fresh Belgian Special Beers. Specific phenolic extraction enabled us to quantify eight volatile phenols (**1**−**8**) in fresh beers (Table 2). Identification was confirmed by mass spectrometry (coincidence of the main m/z values with those of commercial references and entries in the NIST library), capillary column (CP-Sil 5 CB MS) retention indices, and odors. All of the phenols were perceived at the sniffing port in the three beers investigated by AEDA, with descriptors such as burned, dentist, roasted, coffee, clove, vanilla, and smoked. Analytical results revealed different phenolic profiles according to the style of beer.

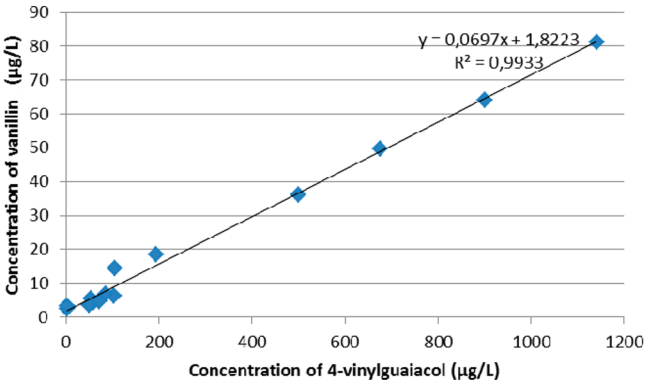

Figure 2. Correlation between the 4-vinylguaiacol and vanillin concentrations (μg/L) in fresh beers.

4-Vinylphenol, 4-Vinylguaiacol, Vanillin, and Apocynol. As shown in Table 2, the level of 4-vinylguaiacol and its analogues was not linked to the style of beer but rather to the yeast strain used (POF+ phenotype confirmed in Table 1 when data available). For instance, the blond BL1 and its organic version, BL2 (fermented with the same yeast), as well as W1 (a traditional Belgian white beer) proved to contain large amounts of all ferulic and coumaric acid-derived phenols. Up to 504.8 μg/L 4-vinylphenol, 1724.6 μg/L 4-vinylguaiacol, and 113.8 μg/L vanillin were evidenced, to be compared with the odor thresholds determined as 170, 125, and 50 μg/L, respectively. Among the amber beers investigated, AM2 reached 330.9 μg/L 4-vinylphenol, 375.8 μg/L 4-vinylguaiacol, and 27.4 μg/L vanillin, whereas AM3 gave rise to very low levels (8.2, 18.7, and 2.3 μg/L, respectively). One brown beer (BR2) was also characterized by high concentrations of 4-vinylphenol and 4-vinylguaiacol (570.3 and 700.6 μg/L, respectively). A nice correlation was observed between the 4-vinylguaiacol and vanillin concentrations in the different beers, suggesting that vanillin could arise through 4-vinylguaiacol oxidation (Figure 2). Apocynol, which results from the addition of a water molecule on 4-vinylguaiacol, was detected only as traces in all

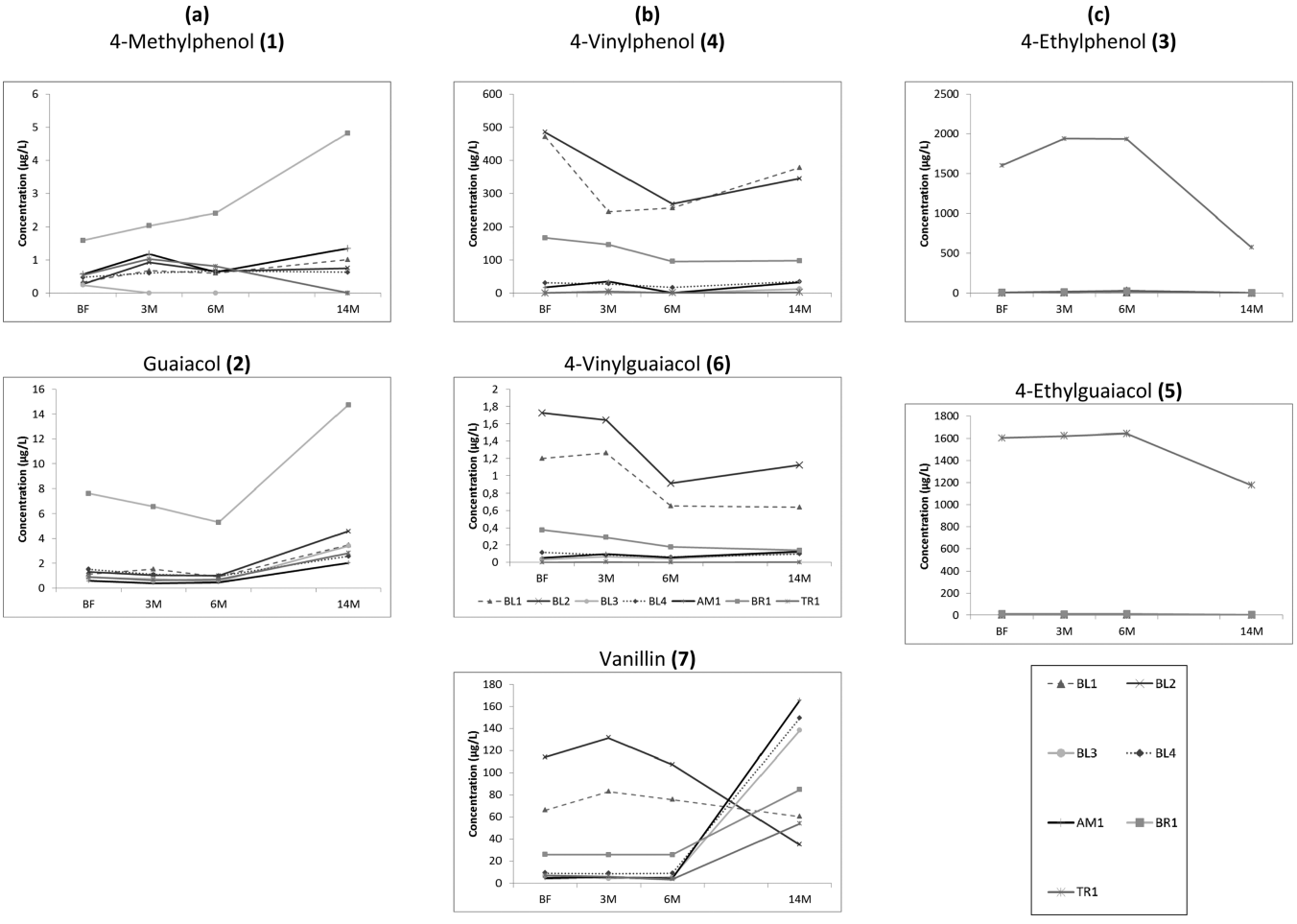

Figure 3. Fate of (a) 4-methylphenol and guaiacol, (b) 4-vinylphenol, 4-vinylguaiacol, and vanillin, and (c) 4-ethylphenol and 4-ethylguaiacol (μg/L) during beer aging.

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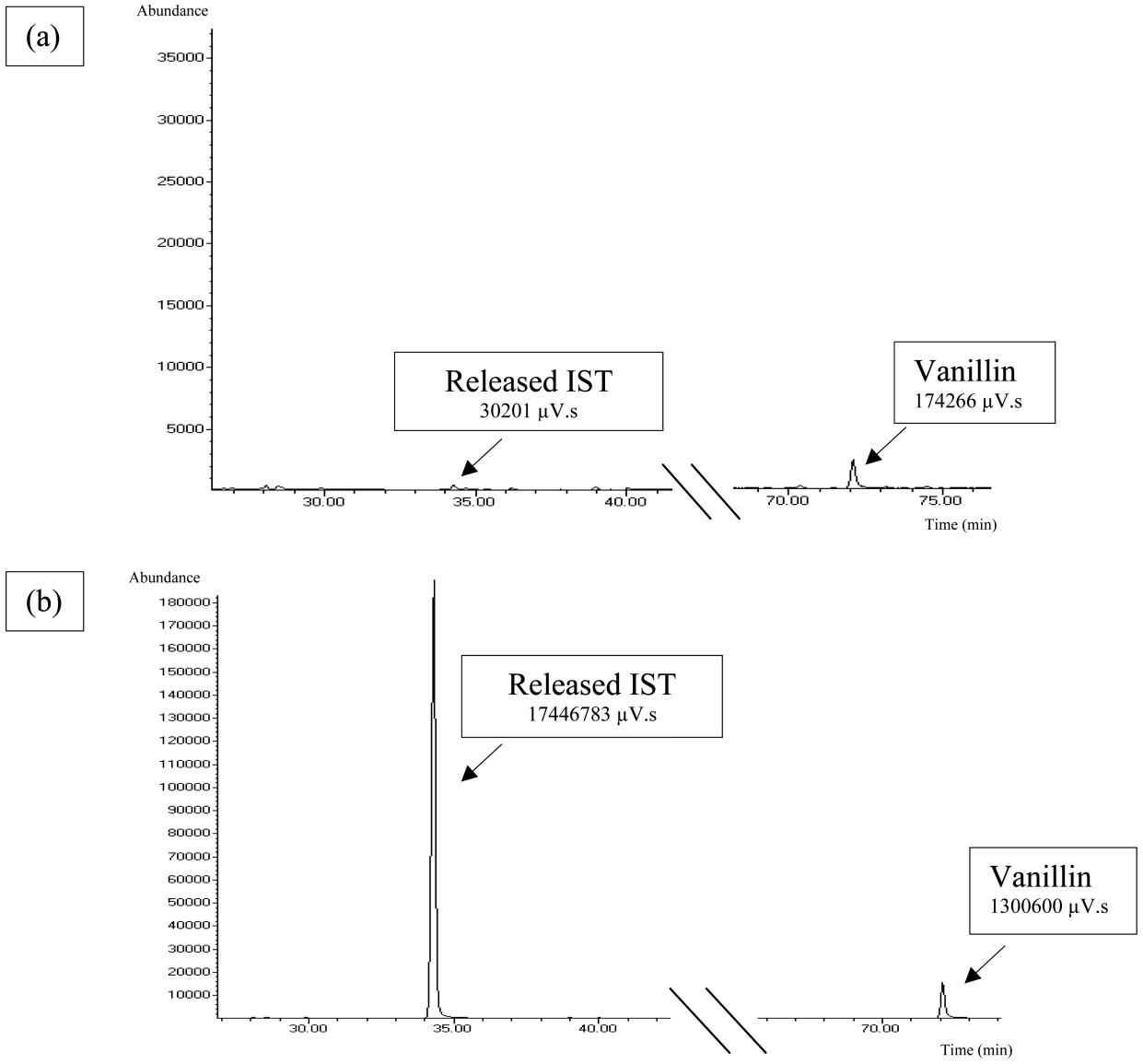

Figure 4. GC-MS-SIM chromatograms of (a) fresh BR1 untreated and (b) fresh BR1 treated with β-glucosidase.

beers. 4-Vinylguaiacol and vanillin were detected at the highest concentrations in BL2, for which organic malts were used. Very recently, Melotte et al.[28] found organic malts to contain higher amounts of ferulic acid (not true for cinnamic acid and its decarboxylated analogue, vinylphenol).

4-Ethylphenol and 4-Ethylguaiacol. Only TR1 showed high levels of 4-ethylphenol (1601 μg/L) and 4-ethylguaiacol (1603 μg/L) (Table 2). After hydroxycinnamic acid decarboxylation to vinylphenols (use of POF(+) yeasts for primary fermentation), the vinylphenol reductase activity of *Brettanomyces* yeasts (added only for secondary fermentation and bottle refermentation) allowed biotransformation of 4-vinylguaiacol and 4-vinylphenol. The residual 4-vinylguaiacol concentration was 0.5 μg/L in TR-1 and above 18 in all 11 other beers. The key role of *Brettanomyces* for reducing 4-vinylguaiacol into 4-vinylphenol was recently confirmed by Collin et al.,[29] who found only traces of 4-ethylguaiacol in TR1 before secondary fermentation ($\leq$2 μg/L). It steadily increases, in the presence of *Brettanomyces*, during the second part of maturation and through bottle refermentation, according to the pitching rate.

Guaiacol and 4-Methylphenol. The brown beers BR1, BR2, and BR3 contained the highest amounts of guaiacol (3.5−9.7 versus 0.4−0.9 μg/L in amber beers) and 4-methylphenol (1.1−3.8 versus 0.2−0.6 μg/L). Chocolate malts were used for all three of these beers, characterized by typical roasted and chocolate odors.[30,31]

Volatile Phenols in Aged Belgian Special Beers. The fate of phenols was monitored in seven beers for 14 months.

Guaiacol, described above as particularly relevant in fresh brown special beers made with chocolate malts, showed a significant concentration rise in all beers after 6 months (reaching 14 μg/L in BR1 after 14 months, Figure 3a). To a lesser extent, this was also the case of 4-methylphenol, which reached 4.9 μg/L in brown beer BR1 after 14 months. With their respective individual odor thresholds of 70 and 20 μg/L, guaiacol and 4-methylphenol could affect the organoleptic properties of aged dark beers. Acid hydrolysis of glycosides is suspected to gradually release these two compounds.

In contrast, 4-vinylphenol and 4-vinylguaiacol proved to be degraded over the first 6 months of storage (Figure 3b), particularly in BL1 and BL2. Oxidation of the double bond to

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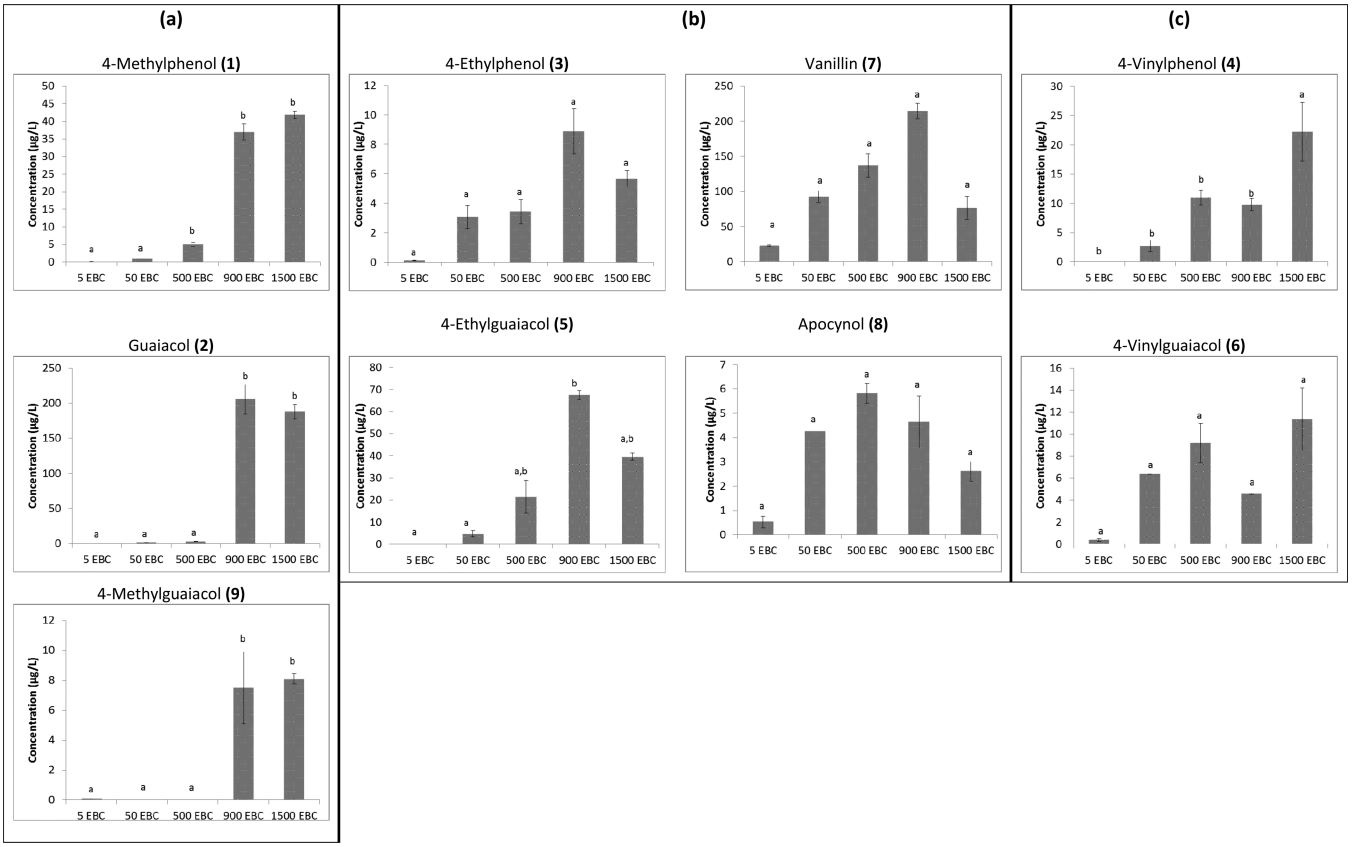

Figure 5. Concentrations (μg/L) of (a) 4-methylphenol, guaiacol, and 4-methylguaiacol; (b) 4-ethylphenol, 4-ethylguaiacol, vanillin, and apocynol; and (c) 4-vinylphenol and 4-vinylguaiacol in EBC laboratory worts, according to the color. Values sharing no common letter are significantly different ($p < 0.05$).

aldehyde might partly explain this evolution, as suggested in five beers by the strong increase in vanillin concentration between 6 and 14 months (Figure 3b). Yet vanillin degradation was observed in sample BL2, characterized by higher levels of both 4-vinylguaiacol and vanillin before aging. Hence, hydrolysis of glycosides most probably also partly contributes to the strong increase in vanillin concentration in BL3, BL4, AM1, BR1, and TR1 (up to 180 μg/L). From a sensory standpoint, these five beers reached the vanillin threshold value only after aging, whereas in BL2 the level of this active constituent of fresh beer fell below its threshold value during storage. BL1 was the only sample in which aldehyde oxidation was nearly balanced by 4-vinylguaiacol oxidation and/or glycoside hydrolysis.

To roughly assess if malt or hop vanillin glucosides could survive through the brewing process to be further hydrolyzed in beer (lower pH than wort), an enzymatic assay was applied on a fresh beer (BR1). As depicted in Figure 4, a β-glucosidase treatment applied on the XAD-2 glucoside extract did release 0.2 mg/L IST equivalent of vanillin. Complementary analyses will be needed to correlate the accurate concentrations of vanillin glucoside in each beer to the amount of free vanillin released through aging. The assay could be also optimized to quantify traces of other suspected glucosides (e.g., guaiacol and 4-methylphenol).

The hydrogenated derivatives 4-ethylphenol and 4-ethylguaiacol found in TR1 were also degraded between 6 and 14 months of aging (Figure 3c). Due to the presence of *Brettanomyces* yeasts in this Trappist bottled beer, 4-ethylphenol was, however, still synthesized during the first 3 months of storage (vinylphenol bioreduction).

Volatile Phenols in EBC Laboratory Worts Produced with Five Different Malts. The origin of guaiacol and 4-methylphenol in dark beers was investigated by analyzing worts produced with five differently colored malts (color ranging from 5 to 1500 °EBC; laboratory worts brewed with 50% special malts; values given for 100%). As depicted in Figure 5a, our results confirmed that high levels of 4-methylphenol (37−42 μg/L) and guaiacol (188−205 μg/L) are specific to the use of torrefied malts ($\leq$5 μg/L 4-methylphenol and $\leq$3 μg/L guaiacol for all other worts). The high temperatures applied during roasting (>250 °C) most probably allow thermal degradation of either free hydroxycinnamic acids or lignins. Also to be mentioned is the presence of 7−8 μg/L 4-methylguaiacol in both worts made with roasted malts only. Taking into account that <10% of torrefied malts are usually used by brewers for special beers, the concentration of this compound was logically too low to be detected in the above-described commercial beers (compound **9** in Table 2). The other phenols are not necessarily found in higher amounts in congress worts made with torrefied malts. Figure 5b shows a similar pattern for four phenols (4-ethylphenol, 4-ethylguaiacol, vanillin, and apocynol): a very low level in pale malt, a maximum concentration in malts of intermediate color, and again a lower level in 1500 °EBC malt. In the case of 4-vinylguaiacol, caramel and torrefied malts can bring similar concentrations (e.g., 500 and 900 °EBC in Figure 5c). For 4-vinylphenol, only the black malt shows a significantly higher concentration.

In conclusion, because guaiacol and 4-methylphenol were always found under 3 μg/L in EBC laboratory worts made with

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caramel malt or pale malts, we suggest that these compounds, especially guaiacol, might be used as markers of torrefied malts.

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Notes

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ABBREVIATIONS USED

EBC, European Brewery Convention; GC-MS, gas chromatography−mass spectrometry; GC-O, gas chromatography−olfactometric detection; AEDA, aroma extract dilution analysis; FD, dilution factor; NIST, National Institute of Standards and Technology; *m/z*, mass-to-charge ratio; POF, phenolic off-flavor

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