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Decrease of 4-Vinylguaiacol during Beer Aging and Formation of Apocynol and Vanillin in Beer

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In this study the decrease of 4-vinylguaiacol (4VG) during beer aging was investigated and the products that arise from it were identified. Two compounds, vanillin and apocynol, were identified in beer model solutions after forced aging and in naturally aged beers by GC-MS and HPLC-ECD analyses. Both account for up to 85% of the decrease of 4VG. Only in the presence of substantial amounts of oxygen in the bottle headspace was vanillin detected. Apocynol [4-(1-hydroxyethyl)-2-methoxyphenol] was found to be the main degradation product, and its formation was shown to be highly dependent on the beer pH. Because both apocynol and vanillin have a clear vanilla-like aroma, the decrease of 4-vinylguaiacol during beer aging might impart a shift from a clove-like aroma in fresh specialty beers (such as wheat beers and other top-fermented blond or dark ales) to a sweeter, more vanilla-like flavor impression of aged specialty beers.

KEYWORDS: 4-Vinylguaiacol; beer aging; phenolic flavor; apocynol; vanillin

INTRODUCTION

Volatile phenols have long been recognized as important flavor contributors to the aroma of nonalcoholic beverages such as fruit juices and coffee (*1, 2*) as well as alcoholic drinks such as beer, wine, sherry, and whiskey (*3–6*). The two main flavor-active volatile phenols in beer are 4-vinylguaiacol (4-vinyl-2-methoxyphenol) (4VG) and 4-vinylphenol (4VP). The presence of these volatile phenolic compounds is considered to be undesirable when present in excessive concentration in bottom-fermented pilsner beers. Hence the term "phenolic off-flavor" (POF) (*7*) is attributed to beers with a strong medicinal, clove-like aroma. Despite being historically catalogued as an off-flavor, these compounds are known to be essential flavor contributors to the characteristic aroma of Belgian white beers (made with unmalted wheat), German Weizen beers (made with malted wheat) (*8*), and Rauch (smoked) beers. However, also in many other top-fermented blond and dark specialty beers, the phenolic flavor is essential for the overall flavor perception. 4VP and 4VG are the decarboxylation products of the phenolic acids *p*-coumaric acid (4-hydroxycinnamic acid) and ferulic acid (4-hydroxy-3-methoxycinnamic acid), respectively. Phenolic acids (i.e., hydroxycarboxylic acids with phenolic hydroxyl groups), more specifically hydroxycinnamic acids, are mainly associated with polysaccharides in the plant cell wall. In cereal grain, they are mainly esterified with arabinoxylans. Arabinoxylans are important structural carbohydrates in the husk, pericarp, aleurone, and endosperm in cereal grains. The relatively flavor-inactive phenolic acids can be decarboxylated to the highly flavor-active volatile phenols 4VP and 4VG in two ways (*9*): (1) by thermal impact during high-temperature treatments in beer production processes such as wort boiling, whirlpool holding, and pasteurization or (2) by enzymatic decarboxylation during fermentation by phenylacrylic acid decarboxylase activity of top-fermenting yeasts strains (Pad1-enzyme) (*10*) or by phenolic acid decarboxylase activity of contaminating micro-organisms (*9, 11*). 4VG is also a key flavor compound in coffee (*1*). The 4VG content in Robusta coffee is reported to be much higher than that in Arabica coffee. It might be responsible for the smoky phenolic odor note, which is more intense in the Robusta coffee brew (*2*). Vinylphenols have been reported as potential off-flavor compounds in other nonalcoholic drinks such as apple juice (*12*) and orange juice (*13–19*), where they impart an old or rotten fruit aroma. They also contribute to the aroma of various other alcoholic drinks such as whiskey (*6*) and sherry (*5*). In wine, both vinylphenols (4VP and 4VG) and their ethyl analogues (4-ethylphenol and 4-ethylguaiacol) have been detected and are important contributors to wine aroma (*3, 20*).

Beer aging is considered to be a major quality problem because the aging flavors are mostly experienced as unpleasant. The development of typical aging flavors during beer storage is linked to the Maillard reaction, the formation of linear aldehydes, ester formation, ester degradation, acetal formation, etherification, and the degradation of hop bitter compounds. Most research on beer aging focused on lager beers. Consequently, the aging process of top-fermented specialty beers is less understood. However, profound differences in the nature of aging between lager and specialty beers have been found. Most of them have been attributed to an increased Maillard reaction in specialty beers (*21, 22*). Because 4VG can be listed as one of the most important flavor compounds in many top-fermented specialty beers, reaching up to 9 times the flavor threshold (*23*), several strategies to optimize its concentration in fresh beer have been postulated (*9, 24, 25*). However,

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McMurrough et al. (*4*) found that there is an appreciable temperature-dependent decrease of 4VG during beer aging, where it is transformed into currently unidentified compounds. Consequently, the decrease of 4VG during beer aging and storage will lead to a diminished perception of the fresh beer flavor, which can be considered typical for the aging process of top-fermented beers. In wine research, the decrease of 4VP and 4VG has been extensively studied and has been partly attributed to the slow acid-catalyzed addition of ethanol, yielding 4-(1-ethoxyethyl)phenol and 4-(1-ethoxyethyl)guaiacol (*26*). It has also been suggested that 4VG might undergo oxidation to vanilla-like compounds (*5*) or oligomerization. Vinylphenols can undergo a cycloaddition with anthocyanins, yielding red-pigmented pyranoanthocyanins (e.g., malvidin-3-*O*-glucoside-4-vinylguaiacol) in wine (*27*−*30*). When wine is stored on yeast lees, volatile phenols may also diminish by the adsorption to the yeast cell membrane (*31*). The objectives of this study were to (1) investigate the decrease in 4VG concentration during beer storage and aging; (2) study the effect of temperature, pH, and oxygen on the degradation rate; and (3) identify the main degradation products that arise from it.

MATERIALS AND METHODS

Quantification of Volatile Phenols, Apocynol, and Vanillin by HPLC-ECD. Quantification of volatile phenols, apocynol [4-(1-hydroxyethyl)-2-methoxyphenol] and vanillin in beer model solutions and beer was performed by HPLC-ECD as described by Vanbeneden et al. (*32*). Before injection, all samples were filtered through 0.45 μm regenerated cellulose syringe filters (Alltech, Deerfield, IL) into autosampler vials and frozen at −18 °C until analysis. For the quantification of apocynol and vanillin, samples (5.0 mL) were extracted three times with 10 mL of ethyl acetate (Acros Organics, Geel, Belgium). After vacuum evaporation of the combined ethyl acetate fractions to dryness at 35 °C, the samples were dissolved in 5 mL of methanol prior to HPLC analysis. The mobile phase composition was $H_2O/CH_3OH/H_3PO_4$ (745:245:10, v/v/v) using a flow rate of 1.0 mL/min. The potential of the working electrode was set at +1200 mV versus Ag/AgCl. Peak areas were analyzed with the Chromeleon chromatography management system version 6.5 (Dionex). The relative standard error (coefficient of variation) was <2.0% for the quantification of the volatile phenols 4VG and 4VP and <10.0% for the quantification of apocynol and vanillin. The latter was somewhat higher due to the necessity to extract the samples before analysis.

Decrease of Volatile Phenols during Beer Aging. The behavior of the volatile phenols 4VG and 4VP during beer storage and aging was studied in two commercial blond specialty beers. The beers were obtained after bottling from two different breweries and were kept at 20 °C for 40 weeks. Samples were collected regularly for the quantification of 4VP and 4VG. Both beers were produced with Pad1(+) top-fermenting yeast strains during the main fermentation and were bottle conditioned by adding extra yeast and sugar during the bottling process (a process known as "Kräusening" or "bottle refermentation"). The first beer (6.9% v/v alcohol; 12 EBC, i.e., beer color as specified by the European Brewing Convention) initially contained 0.61 mg/L 4VP and 1.37 mg/L 4VG, whereas the second blond specialty beer (9.3% v/v alcohol; 14 EBC, pH 4.22) initially contained 0.65 mg/L 4VP and 2.71 mg/L 4VG.

In a second experiment, 2 mg/L 4VG was added to a pilsner beer. Before bottle capping, the headspace was flushed with either oxygen or carbon dioxide. Samples were incubated at 4, 20, 40, and 60 °C for 12 weeks. Samples kept at 20 °C (natural aging) were monitored for 1 year. After the bottle headspace had been flushed with oxygen, the beer contained 3.5−4.0 mg/L dissolved oxygen, whereas the total in-pack oxygen in the bottle reached 12−12.5 mg/L.

Forced Aging of 4VG in a Beer Model Medium. To facilitate the detection and identification of novel compounds arising from 4VG during beer storage, forced aging experiments were conducted in beer model solutions. A beer model solution was prepared to reflect the concentration of the various major beer compounds, pH, nitrogen content, and ethanol content of beer according to the method of Sadosky et al. (*33*). The solution was prepared by adding 500 mg of sodium azide, 6.962 mg of potassium phosphate, 2.034 mg of sodium chloride, 2.281 mg of calcium chloride, 7.097 mg of magnesium sulfate, 4.480 mg of proline, 2.590 mg of valine, 2.250 mg of alanine, and 10 g of bovine serum albumin to 9 L of water with stirring for 1 h. Absolute ethanol (600 mL) was added, and the final volume was brought to 10 L with water. The solution was then adjusted to pH 4.2 by the addition of phosphoric acid. 4VG was added at a concentration of 3.5 mg/L. Samples were force aged at 60 °C for 3 weeks. Different experimental setups were investigated: the headspace was flushed with oxygen or carbon dioxide, the ethanol content was varied between 5 and 20% v/v, and a broad pH range was examined by setting the pH at 4, 7, or 10. Additionally, more realistic beer pH values were investigated at pH 3.4, 3.7, 4.0, 4.3, and 4.6.

Identification of Unknown Compounds by GC-MS and HPLC-ECD. Samples were extracted three times with ethyl acetate. The combined fractions were evaporated to dryness and redissolved in methanol. Samples (1 μL) were injected on a Trace GC Ultra (Thermo, Waltham, MA) equipped with a split/splitless injector, which was used in splitless mode. The temperature of the injector was set at 250 °C. The carrier gas was helium at a flow rate of 1.5 mL/min. Compounds were separated on an Rtx-5 Sil MS (60 m × 0.25 mm i.d., 1.0 μm film thickness) (Restek, Bellefonte, PA). The oven temperature profile was as follows: 2 min at 60 °C, raised to 100 at 20 °C/min, to 220 at 15 °C/min, and to 280 at 20 °C/min, and a final hold of 5 min at 280 °C. Electron impact mass spectra were recorded at 70 eV (full scan with a mass range from 35 to 350 *m/z*) on a Dual Stage Quadrupole (DSQ) MS (Thermo, Waltham, MA). Results were compared with those of pure reference compounds of both apocynol and vanillin. Analytical grade vanillin was obtained from UCB (Brussels, Belgium). A sample of pure reference compound of apocynol was kindly provided by Prof. Ilkka Kilpelainen and Dr. Pirkko Karhunen from the University of Helsinki (Department of Chemistry, Laboratory of Organic Chemistry). They synthesized the apocynol by the reduction of acetovanillone with $NaBH_4$ in 56% aqueous ethanol as described by Bailey et al. (*34*). Apocynol is characterized by the following data (*35*): ^{1}H ($CDCl_3$) δ 1.47−1.49 (d, 3H, *CH₃*), 1.97 (br s, 1H, *OH*), 3.90 (s, 3H, *OCH₃*), 4.81−4.85 (q, 1H, *CH*), 5.58−5.60 (m, 1H, *OH*), 6.84−6.94 (m, 3H, *aromatic*); ^{13}C NMR ($CDCl_3$) δ 25.06 (CH_3), 55.88 (OCH_3), 70.31 (*HCOH*), 107.94, 114.11, 118.30, 137.90, 144.96, 146.57 (*aromatic*); GC-MS, *m/z* (%) 168 (M^+,62), 153 (99), 135 (17), 125 (39), 110 (13), 93 (100), 77(16), 65 (44), 53 (14), 53 (14), 43 (53), 39 (15). Extracted samples were spiked and injected on a HPLC-ECD system with a C18 column (see above).

RESULTS AND DISCUSSION

Decrease of Volatile Phenols during Beer Aging. Two commercial blond specialty beers were aged at 20 °C for 40 weeks. The behavior of the 4VG and 4VP contents of both beers during beer aging is presented in **Figure 1**. In the first beer, an initial, although small, increase in both 4VP and 4VG contents could be noted (0.08 and 0.22 mg/L) due to Kräusening with a highly Pad1(+) yeast strain. After the initial increase, the 4VP and 4VG contents decreased continuously with a $t_{1/2}$ of 34 weeks. In the second beer, the 4VP and 4VG immediately decreased, reaching half of the initial value after 31 weeks. The absence of the initial increase of the 4VG concentration in the second beer can be due to two reasons: (1) the bottle conditioning step was performed with a yeast strain different from that used during the main fermentation and this second yeast strain was Pad1(−) or (2) after the main fermentation, all precursor molecules (ferulic acid) were already converted to 4VG. The flavor threshold of 4VG in beer has been reported to be 0.3 mg/L (*36*). Both beers initially contained highly flavor-active amounts of 4VG of 5.3 and 9.0 flavor units, respectively. One flavor unit is defined as the concentration divided by the flavor

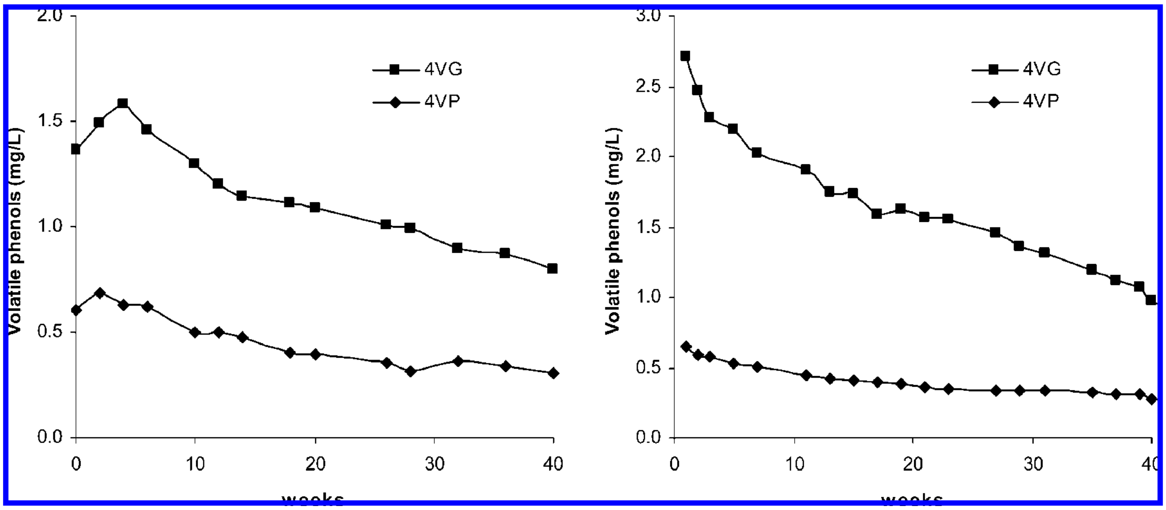

Figure 1. Behavior of the volatile phenols 4VP (mg/L) and 4VG (mg/L) during natural beer aging in two commercial blond specialty beers. Both beers were obtained after bottling from two different breweries and were kept at 20 °C for 40 weeks. Samples were collected regularly for the quantification of 4VP and 4VG. The first beer (left) (6.9% v/v alcohol; 12 EBC) initially contained 0.61 mg/L 4VP and 1.37 mg/L 4VG, whereas the second blond specialty beer (right) (9.3% v/v alcohol; 14 EBC, pH 4.22) initially contained 0.65 mg/L 4VP and 2.71 mg/L 4VG.

Table 1. Evolution of 4VG (Milligrams per Liter) during Aging of a Pilsner Beer with 2 mg/L Supplemented 4VG at 4, 20, 40, and 60 °C with Carbon Dioxide Flushed Headspace

temp (°C)	start	6 weeks	12 weeks
4	2.13	2.11	2.06
20	2.13	1.94	1.74
40	2.13	1.41	1.14
60	2.13	0.66	0.33

Table 2. Evolution of 4VG (Milligrams per Liter) during Forced Aging (60 °C) of a Pilsner Beer with 2 mg/L Supplemented 4VG and the Bottle Headspace Flushed with either Oxygen or Carbon Dioxide

	start	2 weeks	6 weeks	12 weeks
60 °C + CO_2	2.13	1.41	0.66	0.33
60 °C + O_2	2.13	0.46	0.33	0.20

threshold and indicates the difference in concentration of a particular compound that can be identified. After 40 weeks at 20 °C, the 4VG concentration decreased by several flavor units to 2.7 and 2.9 flavor units, respectively, indicating that the decrease of the volatile phenol concentration during beer conservation can have a profound impact on beer flavor.

In a second experiment, a beer fortified with 4VG was aged at different temperatures and with either oxygen or carbon dioxide in the headspace. Results are represented in **Tables 1** and **2**. As can be seen from **Table 1**, the 4VG content of beer decreased more rapidly when the temperature during beer aging was higher. When the headspace was flushed with oxygen, the final 4VG concentration was significantly lower than that when the headspace was flushed with carbon dioxide (**Table 2**). It seems that, at least partly, oxygen might act as a catalyst (radical reactions) or as a reactant. Possible explanations for the decline of the 4VG concentration during beer aging are reduction or oxidation of the double bond, oligomerization, bonding with polyphenols, formation of ethoxyethyl phenols, adsorption to yeast lees, or further metabolization by yeast. Because 4VG also declined during aging of the pilsner beer (without bottle refermentation), the latter two cannot solely account for the observed losses of volatile phenols during beer aging. Moreover, no metabolization of 4VG by *Saccharomyces cerevisiae* has been reported so far. When *Brettanomyces* spp. are involved, the loss of 4VG can be due to the reduction of 4VG to 4-ethylguaiacol (*20*).

Forced Aging of 4VG in Beer Model Medium and Identification of Vanillin. To facilitate the detection and identification of novel compounds arising from 4VG during beer storage, forced aging experiments were conducted in beer model solutions. A beer model solution was forced aged at 60 °C for 3 weeks, and the effect of oxygen, pH, and ethanol was investigated. The initial blank sample (3.5 ppm of 4VG) contained a minor impurity at a retention time (RT) of 15/16 min (0.15 nA × min).

After 3 weeks at 60 °C, 4VG concentrations decreased, although different effects of oxygen content, pH, and ethanol content could be seen. With regard to the oxygen content, the sample with the oxygen-flushed headspace contained less 4VG than the sample with the carbon dioxide-flushed headspace (1.90 and 2.36 mg/L, respectively). In both samples, a peak arose at a RT of 9 min. The peak area was larger in the carbon dioxide-flushed sample than in the oxygen-flushed sample (1.88 and 1.33 nA × min, respectively). Also, the "impurity" peak grew during the forced aging: 0.18 and 0.54 nA × min for the carbon dioxide and the oxygen samples, respectively. The peak at 15/16 min was identified as vanillin by spiking the sample with pure reference compound and varying the polarity of the mobile phase on the HPLC-ECD system. The identity was confirmed by GC-MS analysis by injection with pure reference vanillin and by comparison with the NIST library. Vanillin is one of the possible oxidation products of 4VG. This explains why its concentration in the oxygen-containing sample is higher than in the carbon dioxide-flushed sample.

The effect of the pH on the decline of 4VG during forced aging was investigated at pH 4, 7, and 10. The pH had a profound effect on the degradation rate of 4VG. At pH 10, the final 4VG content after forced aging was the lowest (0.18 mg/L), and vanillin was the most abundant compound formed (1.70 nA × min). At pH 7, the final 4VG content remained the highest of the three pH values examined (2.25 mg/L). At pH 4, the final 4VG concentration (0.90 mg/L) was lower than that at pH 7, but higher than that at pH 10. The most important degradation product formed at pH 4.0 was the unknown compound at RT 9 min (2.43 nA × min). At higher pH values, the oxidation of

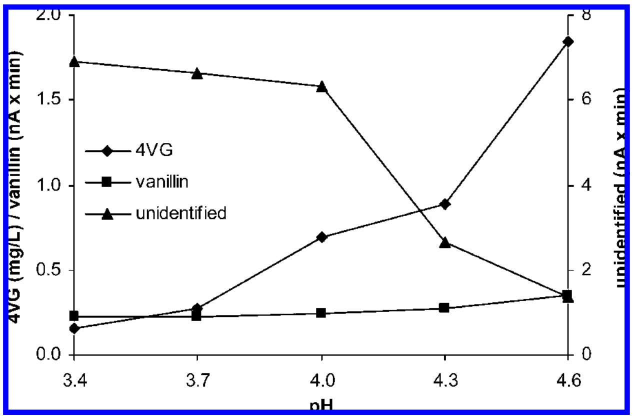

Figure 2. Final amounts of 4VG (mg/L), vanillin (nA × min), and unidentified compound (nA × min) after forced aging (60 °C, 3 weeks) of a beer model solution at different pH values initially containing 3.5 mg/L 4VG.

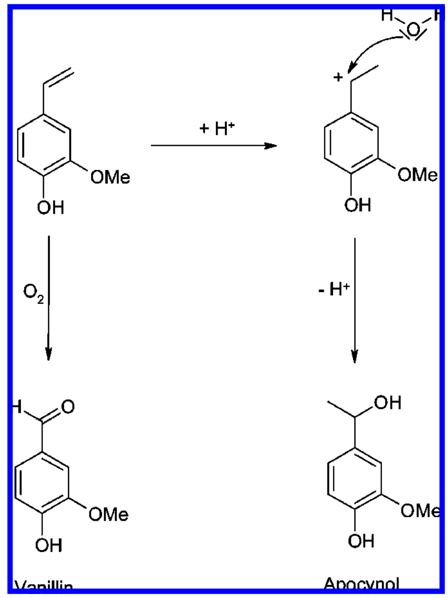

Figure 3. Proposed degradation patterns of 4VG during beer aging: oxidation leading to vanillin and hydration leading to apocynol.

4VG seemed to be the most important route of 4VG decline. The formation of the unknown compound at a RT of 9 min was likely to be the result of an acid-catalyzed reaction. At neutral pH, 4VG was less affected than at high or low pH values.

The degradation of 4VG was also investigated at more realistic beer pH values (3.4, 3.7, 4.0, 4.3, and 4.6). The results for the 4VG content (mg/L), the peak area corresponding with vanillin (nA × min), and the peak area corresponding to the unknown compound (nA × min) are depicted in **Figure 2**. It can be clearly seen that the decline of the 4VG concentration was highly dependent on the pH. Even small changes in pH gave rise to highly differing final 4VG concentrations. The higher final 4VG concentration at higher pH values seemed to be mostly due to the lower amount of the unidentified compound being formed, confirming that it must be an acid-catalyzed reaction. The formation of vanillin by the oxidation of 4VG increased slightly with increasing pH values, but it was less affected within the examined pH range.

No effect of the ethanol content (between 5 and 20% v/v) on the degradation of 4VG was observed. This made it unlikely that ethanol would be involved in the decrease of 4VG during beer aging. Remarkably, when 4VG was forced aged in pure ethanol, no peak at a RT of 9 min was observed. This confirmed that ethanol was not involved in the formation of the unknown compound.

Identification of Apocynol by GC-MS and HPLC-ECD. The mass spectrum of the second unknown compound (RT of 9 min) was characterized by the following *m/z* ions (%): 153 (100), 168 (M^+, 86), 93 (84), 125 (64), 65 (53), and 43 (30). The molecular weight of the second unknown compound was found to be 168, leading to the hypothesis that it might have been formed by the reaction of 4VG (MW 150) with water (MW 18). If a hydration of the 4VG molecular structure occurred, it was most likely to occur on the double bond of the vinyl side chain. Hydration reactions are favored at acidic pH because the initial rate-limiting step requires the addition of a proton to the less substituted carbon of the double bond. The initial acid-catalyzed addition of a proton to the double bond is favored by the presence of oxygenated electron-donating groups on the aromatic ring of 4VG. Moreover, the carbocation formed is stabilized by resonance. In the second step, a water molecule binds to the more highly substituted carbon. The addition follows Markovnikov's rule implicating the hydroxyl group adds to the carbon with the highest number of C−C bonds, forming the most stable carbocation. The attack of water on the carbocation should gain the compound presented in **Figure 3**. The polarity of the suggested product also corresponded with its relative position in the HPLC-ECD chromatograms between the other vanillic compounds. Literature on lignin and wood research (*35, 37, 38*) led to the identification of apocynol, also called 4-(1-hydroxyethyl)-2-methoxyphenol, 1-guaiacylethanol, 4-(1-hydroxyethyl)guaiacol, or α-methylvanillyl alcohol. Analysis of a sample of pure reference compound confirmed the presence of apocynol in the extracted samples. Both vanillin and apocynol have a clear vanilla-like flavor. A proposed fragmentation pattern of apocynol is shown in **Figure 4**. The fragmentation pattern that gives rise to the *m/z* 125 fragment is based on the proposed fragmentation pattern characteristic of an hydroxyl group on an aromatic ring according to McLafferty et al. (*39*). The mechanism involves the elimination of oxygen and its adjacent ring carbon. The *m/z* 125 fragment has also been reported in the mass spectrum of vanillyl alcohol according to the NIST library. Extracted samples spiked with pure reference compounds were also injected on a HPLC-ECD system. Both peaks coeluted regardless of the polarity of the mobile phase obtained by changing the methanol concentration.

Quantification of Apocynol and Vanillin in Aged Beer. Apocynol and vanillin were quantified in aged lager beers, which were supplemented with 2 mg/L 4VG. The bottle headspace was flushed with either carbon dioxide or oxygen. Beers were naturally aged at 20 °C, and samples were taken after 3, 6, and 12 months for analysis of 4VG, apocynol, and vanillin. Values are presented in **Table 3**. 4VG decreased more rapidly when the bottle headspace was flushed with oxygen than with carbon dioxide. When the headspace was flushed with carbon dioxide, no vanillin could be detected apart from a small amount (0.04 mg/L) in the final sample taken after 1 year. In contrast, apocynol increased remarkably during the aging process, reaching 0.83 mg/L after 12 months. When beers were aged without 4VG supplementation, no apocynol or vanillin could be detected, indicating that both originated from the volatile phenol. When the amounts of both vanillin and apocynol were taken into account, >85% of the decrease of 4VG could be explained. When the bottle headspace was flushed with oxygen, the formation of both apocynol and vanillin could be detected. Both accounted for 75% of the decrease of 4VG during the aging process. Because oxygen readily forms reactive radical species,

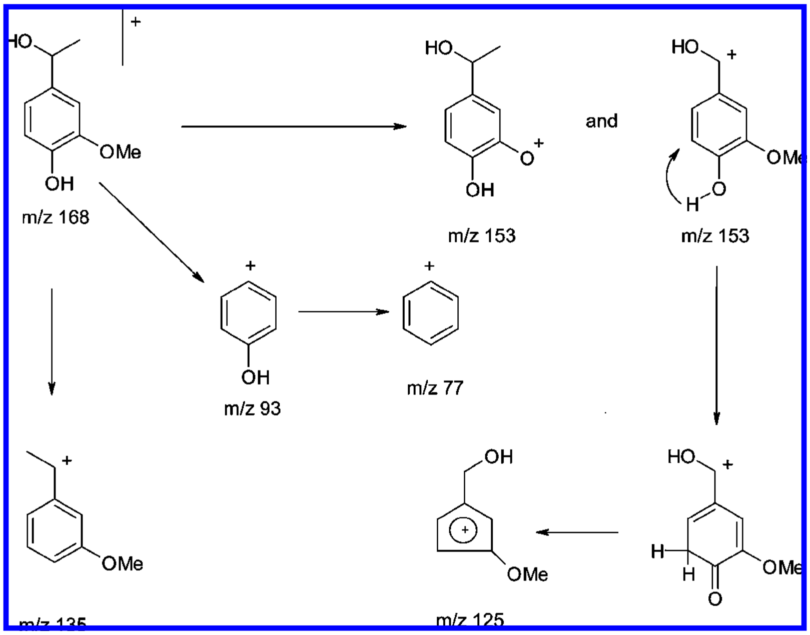

Figure 4. Proposed fragmentation pattern of the molecular ion of apocynol. The fragmentation pattern that gives rise to the *m/z* 125 fragment is based on the proposed fragmentation characteristic of a phenolic ring moiety according to McLafferty et al. (*39*).

Table 3. Evolution of 4VG, Apocynol, and Vanillin during Aging (20 °C) of a Pilsner Beer Supplemented with 2 mg/L 4VG with and without Oxygen in the Bottle Headspace

	4VG (mg/L)	apocynol (mg/L)	vanillin (mg/L)
	Carbon Dioxide-Flushed Headspace		
start	2.13	ND[a]	ND
3 months	1.69	0.36	ND
6 months	1.46	0.44	ND
12 months	1.04	0.83	0.04
	Oxygen-Flushed Headspace		
start	2.13	ND	ND
3 months	1.15	0.28	0.33
6 months	0.89	0.37	0.41
12 months	0.31	0.81	0.59

[a] ND, not detectable.

it is possible that other oxidation products of 4VG were formed in minor amounts. The reaction of 4VG with oxygen leading to vanillin implies that 4VG displays antioxidant activity during beer aging. Concerning the impact on the flavor impression of the beer after 1 year, the decreases in 4VG concentration in the samples with carbon dioxide- and oxygen-flushed headspace were 3.6 and 6.1 flavor units, respectively. Flavor threshold values of vanillin in alcoholic beverages have been reported to vary within a range from 60 μg/L (*40*) to 200 μg/L (*41*). Hence, if initial concentrations of 4VG in the beer are high and some oxygen is present in the bottle headspace, vanillin concentrations can reach flavor threshold values in beer. The flavor threshold of apocynol could not be determined because of the lack of a food grade pure compound.

In conclusion, in this study, the decrease of 4VG during beer aging was investigated, and the products arising from it were identified. Both vanillin and apocynol were identified in beer model solutions after forced aging and in naturally aged beers. Apocynol has, to the best of our knowledge, not yet been reported in beer. Because both apocynol and vanillin have a clear vanilla-like aroma, the decrease of 4VG during beer aging might impart a shift from a clove-like aroma in fresh specialty beers to a sweeter, more vanilla-like flavor impression of aged specialty beers. The reaction mechanisms described in this study contribute to a better understanding of the typical processes underlying the diminishing of the fresh flavor of specialty beers during beer aging and conservation.

ABBREVIATIONS USED

4VG, 4-vinylguaiacol; 4VP, 4-vinylphenol; POF, phenolic off-flavor; Pad1, phenylacrylic acid decarboxylase enzyme; GC-MS, gas chromatography with mass spectromety; HPLC-ECD, high-performance liquid chromatography with electrochemical detection; MW, molecular weight; NIST, National Institute of Standards and Technology; *m/z*, mass to charge ratio.

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