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Ferulic Acid Release and 4-Vinylguaiacol Formation during Brewing and Fermentation: Indications for Feruloyl Esterase Activity in *Saccharomyces cerevisiae*

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The release of ferulic acid and the subsequent thermal or enzymatic decarboxylation to 4-vinylguaiacol are inherent to the beer production process. Phenolic, medicinal, or clove-like flavors originating from 4-vinylguaiacol frequently occur in beer made with wheat or wheat malt. To evaluate the release of ferulic acid and the transformation to 4-vinylguaiacol, beer was brewed with different proportions of barley malt, wheat, and wheat malt. Ferulic acid as well as 4-vinylguaiacol levels were determined by HPLC at several stages of the beer production process. During brewing, ferulic acid was released at the initial mashing phase, whereas moderate levels of 4-vinylguaiacol were formed by wort boiling. Higher levels of the phenolic flavor compound were produced during fermentations with brewery yeast strains of the Pof$^+$ phenotype. In beer made with barley malt, ferulic acid was mainly released during the brewing process. Conversely, 60−90% of ferulic acid in wheat or wheat malt beer was hydrolyzed during fermentation, causing higher 4-vinylguaiacol levels in these beers. As cereal enzymes are most likely inactivated during wort boiling, the additional release of ferulic acid during fermentation suggests the activity of feruloyl esterases produced by brewer's yeast.

KEYWORDS: ***trans*-4-Hydroxy-3-methoxycinnamic acid; 2-methoxy-4-vinylphenol; white beer; Weizen beer; phenolic off-flavor; *PAD1***

INTRODUCTION

Barley malt is usually the major raw material for the production of beer. However, some beer styles are brewed with substantial levels of wheat or wheat malt. Belgian white beers are made with as much as 30−50% of unmalted wheat, whereas brewers use from 50 to 80% of malted wheat for the production of German Weizen beers. In these cereal grains, ferulic acid is the most abundant hydroxycinnamic acid (*1*) and comprises about 0.04−0.06% (w/w dry weight) in barley (*2*, *3*) and 0.05−0.07% (w/w dry weight) in wheat (*4*). The highest levels of this phytochemical were found in the outer layers of barley and wheat, especially in the arabinoxylan-rich aleurone cell walls (*5*, *6*). Generally, ferulic acid monomers as well as different ferulic acid dehydrodimers (*7*, *8*) and cycloaddition products (*9*) are covalently bound to the cell wall. Ferulic acid is reported to be esterified to arabinoxylans (*10*−*12*) and esterified or etherified to lignin surfaces (*13*, *14*). It has been reported that fungal feruloyl esterases are able to release ferulic acid from residues of barley malt (*15*) and wheat (*16*). The release of ferulic acid in wheat bran and brewer's spent grain by feruloyl esterases seems to be enhanced in the presence of main-chain arabinoxylan-hydrolyzing enzymes such as xylanases and arabinases (*15*, *17*, *18*). During malting and brewing, feruloyl esterase activity was found to be native to barley, rather than due to microbial contamination (*19*). The levels of ferulic acid extracted and released during mashing can be controlled and are maximal at a pH value of 5.8 and a mashing temperature of 45 °C (*20*, *21*).

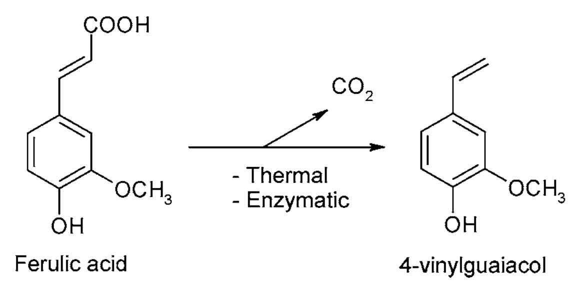

Figure 1. Decarboxylation of ferulic acid to 4-vinylguaiacol.

Throughout the beer production process, ferulic acid can decarboxylate, leading to the formation of 4-vinylguaiacol (**Figure 1**). This compound has a low sensory threshold value and can, even at trace levels, produce a significant phenolic flavor in beer. The threshold of 4-vinylguaiacol is reported to be 0.3 mg/L, whereas its precursor has a much higher threshold value of ∼600 mg/L (*22*). The flavor, often described by the terms medicinal, phenolic, clove-like, or smoky, is appreciated

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in some beer styles such as Belgian wheat beers and German Weizen and Rauch beers (*20*). In most beers, however, the flavor of 4-vinylguaiacol is considered to be undesirable (*23*) and therefore referred to as “phenolic off-flavor” (POF). Ferulic acid can be decarboxylated by thermal decomposition (*24*) during wort boiling (*21*, *25*) and by specific enzymes during fermentation. Hydroxycinnamate decarboxylases are produced by a number of microorganisms; these enzymes are involved in diminishing, transforming, or removing the strong antimicrobial activity of the hydroxycinnamic acids to less toxic metabolites (*26*). In the yeast *Saccharomyces cerevisiae,* a single dominant nuclear gene, designated *PAD1* (alias *POF1*), encodes for ferulate decarboxylase (*27*). This enzyme has been reported not only in some brewing and nonbrewing strains of *S. cerevisiae* (*27*−*29*) but also in other yeast species and wort-contaminating bacteria (*30*−*32*).

Grain type and quality, mashing parameters, yeast condition, fermentation technology, type and size of fermentation vessel, conditioning, and storage are all of importance for the level of 4-vinylguaiacol in beer provided that a Pof^+ yeast strain is used (*20*, *23*, *33*). In general, beers brewed with wheat (*34*) or wheat malt (*23*) appear to generate higher levels of 4-vinylguaiacol than beers brewed with 100% barley malt. At present, few details on the contribution of these alternative raw materials to the phenolic character of certain beer styles are available. Therefore, the main aim of the current study was to investigate the influence of the level of wheat and wheat malt on the release of ferulic acid and the formation of 4-vinylguaiacol during brewing and fermentation with Pof^+ yeast strains.

EXPERIMENTAL PROCEDURES

Materials. Scarlett barley malt was provided by Cargill Malt Division (Herent, Belgium). The wheat cultivars Agato, Beaufort, Claire, Corvus, Drifter, Genghis, Kinto, Legat, Skater, and Zohra were supplied by AVEVE (Landen, Belgium), and Vivant wheat was supplied by Clovis Matton (Avelgem, Belgium). Wheat malt was obtained from Dingemans (Stabroek, Belgium) and Weyermann (Bamberg, Germany). Northern Brewer hops contained 5.1% α-acids (Analytica-EBC).

Brewery strains of *S. cerevisiae* CMBS 1 (lager), CMBS 33 (lager), CMBS 201 (ale), CMBS 203 (ale), CMBS 210 (ale), CMBS 242 (ale), and CMBS 344 (ale) were obtained from the CMBS (Centre for Malting and Brewing Science) yeast collection, Leuven, Belgium.

All reagents were of analytical grade. HPLC grade ferulic acid (*trans*-4-hydroxy-3-methoxycinnamic acid) and 4-vinylguaiacol (2-methoxy-4-vinylphenol) were purchased from Sigma-Aldrich Chemie GmbH, (Munich, Germany).

Methods. *Quantification of Ferulic Acid and 4-Vinylguaiacol.* Ferulic acid and 4-vinylguaiacol levels were determined by HPLC using a method described by McMurrough et al. (*21*). The Dionex (Sunnyvale, CA) DX 500 chromatography system consisted of a Rheodyne (Rohnert Park, CA) model 9125 automatic sample injector (40 μL sample loop), a Dionex GP40 gradient pump, and a Dionex ED40 electrochemical detector. The detector was operated with a glassy carbon working electrode at a potential of +0.9 V versus Ag/AgCl and an output range of 20 nA. The analysis was performed on a 25 cm × 4 mm Nucleosil C18 10 μm column (Machery-Nagel, Düren, Germany) eluted with $H_2O/CH_3OH/H_3PO_4$ (640:350:10, v/v).

Sample Preparation. All samples were protected from light to minimize the photoisomerization reaction to which ferulic acid is susceptible. Wort and degassed beer samples were filtered through 0.45 μm regenerated cellulose syringe filters (Alltech, Deerfield, IL).

Congress Wort Brewing. Standard laboratory Congress wort preparations were performed according to Analytica-EBC (*35*). Barley malt, wheat, and wheat malt were ground in a Buhler-Miag malt mill (Buhler-Miag, Minneapolis, MN) set for EBC fine-grind. In total, 50 g of different levels of barley malt and wheat or barley malt and wheat malt was used for Congress wort brewing (**Table 1**). The time−temperature profile of the mashing process is indicated in **Figure 3**. Samples were acquired at the start of mashing, after 1 and 2 h of extraction and after filtration over a folded filter (MN 614 1/4 32 cm diameter, Macherey-Nagel GmbH). The samples were centrifuged (1400*g*, 5 min), and the supernatant was analyzed for ferulic acid and 4-vinylguaiacol by HPLC.

Wort Boiling. Exactly 200 g of filtered wort was boiled in a glycerol bath (60 min, 106 °C). Unless otherwise stated, 260 mg of hop was

Table 1. Levels of Barley Malt, Wheat, and Wheat Malt Used for Brewing

representation	% barley malt	% wheat	% wheat malt
BM_{100}	100	0	0
$BM_{70}W_{30}$	70	30	0
$BM_{50}W_{50}$	50	50	0
$BM_{40}W_{60}$	40	60	0
$BM_{70}WM_{30}$	70	0	30
$BM_{50}WM_{50}$	50	0	50
$BM_{40}WM_{60}$	40	0	60
WM_{100}	0	0	100

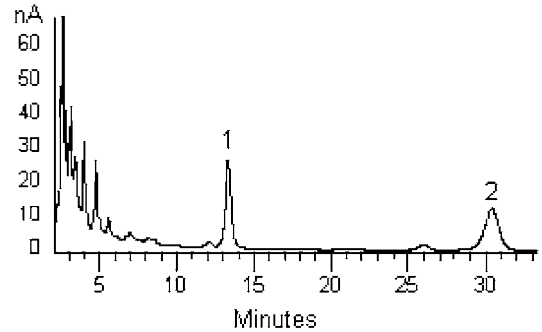

Figure 2. HPLC chromatogram of beer: ferulic acid (1) and 4-vinylguaiacol (2) have isolated peaks with retention times of 13.33 and 30.27 min, respectively.

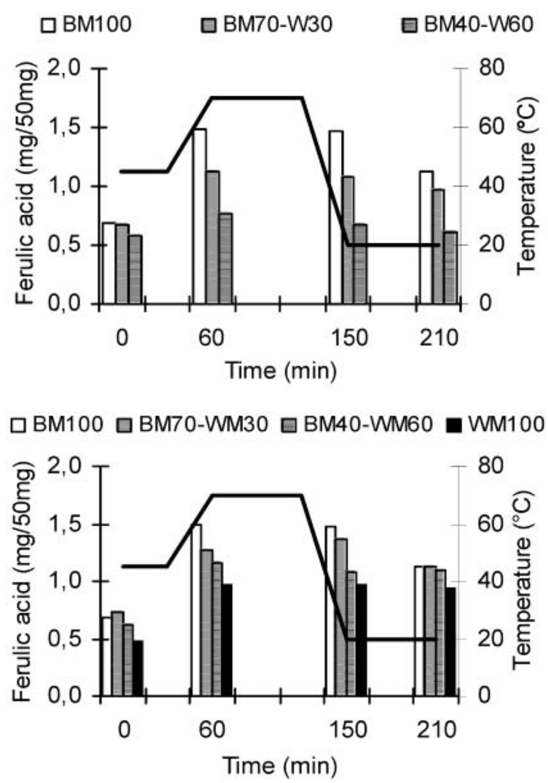

Figure 3. Release of ferulic acid during mashing with barley malt (BM) and wheat (W) or barley malt (BM) and wheat malt (WM). Ferulic acid levels are expressed in milligrams per 50 mg of cereal grains.

Table 2. Ferulic Acid (FA) in Congress Wort Brewed with Different Wheat Cultivars

wheat cv.	FA (mg/L)	wheat cv.	FA (mg/L)
Legat	1.83	Drifter	2.23
Kinto	1.93	Vivant	2.23
Skater	1.94	Beaufort	2.38
Corvus	1.96	Ajami	2.49
Zohra	2.01	Genghis	2.85
Claire	2.14		

Table 3. Formation of 4-Vinylguaiacol (4-VG) during Boiling of Congress Wort

	4-VG (mg/L)		
boiling time (h)	BM_{100}	$BM_{50}W_{50}$	$BM_{50}WM_{50}$
0.5	0.057	0.068	0.073
1	0.146	0.103	0.126
2	0.221	0.193	0.233
3	0.285	0.225	0.304

added to obtain 20 European bitterness units (*35*). Boiled wort was cooled to 20 °C, and the reduction of the wort volume due to evaporation was compensated by the addition of water.

Yeast Propagation. Single isolated colonies of purified yeast strains were inoculated into 100 mL of YPD medium (40 g/L glucose, 20 g/L peptone, 10 g/L yeast extract) and incubated at 27 °C. After 48 h, yeast cells were harvested by centrifugation (1500*g*, 30 min). The supernatant was decanted and the resultant pellet was resuspended in 50 mL of cold water (4 °C). Yeast cell densities were determined with a Thoma counting chamber.

Screening for Pof^+ Phenotype. Eight different commercial brewery yeast strains were screened for the Pof phenotype according to a method described by McMurrough et al. (*21*). The designation of Pof^+ was given to those cultures producing >2.5 mg/L of 4-vinylguaiacol in the test.

Fermentation of Wort. Congress wort or standard 12 °Plato wort, prepared as described by Delvaux et al. (*36*), was boiled for 1 h in glycerol (106 °C), cooled, and subsequently aerated to obtain an oxygen concentration of 8 ppm. The wort was then transferred to fermentation tubes and pitched with 10×10^6 yeast cells per milliliter. The incubation temperature was set at 20 °C. During fermentation, samples were taken 10 cm below the fluid surface and analyzed for ferulic acid and 4-vinylguaiacol and for extract using a density and sound analyzer (DSA-4, A. Paar, Graz, Austria) with an SP-1 autosampler.

Induction or Inhibition of Enzymatic Decarboxylation. Yeast cells (10×10^6/mL) were grown in 500 mL of YPD medium (40 g/L glucose, 20 g/L peptone, 10 g/L yeast extract). Induction and inhibition of the decarboxylation reaction were assessed after supplementation with ferulic acid (final concentration of 0.5, 1, 2, 5, 10, 30, or 50 mg/L). After 4 days of fermentation at 20 °C, samples were analyzed for ferulic acid and 4-vinylguaiacol.

RESULTS AND DISCUSSION

Beer brewed with wheat or wheat malt frequently contains considerable levels of 4-vinylguaiacol, formed by decarboxylation of ferulic acid. To study the influence of wheat and wheat malt on the level of 4-vinylguaiacol and its precursor, brewing experiments with different quantities of these alternative raw materials were conducted (**Table 1**). The release of ferulic acid and the formation of 4-vinylguaiacol were investigated by HPLC (**Figure 2**). In boiled wort and beer, both compounds could be identified, whereas in unboiled wort, only ferulic acid was detectable.

Influence of Wheat and Wheat Malt on Ferulic Acid Release during Mashing. Initially, the effect of the wheat cultivar on the level of ferulic acid in wort mashed with 50% wheat ($BM_{50}W_{50}$) was examined. The ferulic acid concentration was moderately influenced by the cultivar (**Table 2**). The two cultivars providing the most extreme levels of free ferulic acid (Genghis and Legat) were selected for further experiments.

Feruloyl esterase activity has been reported in barley malt (*19*). Moreover, the existence of different barley malt feruloyl esterases has been proposed (*37*, *38*). It was investigated whether these enzymes contribute to an additional release of ferulic acid during mashing with wheat or wheat malt. As unmalted wheat contains only low levels of active enzymes, supplemental barley malt enzymes such as amylases and proteases are required during mashing. In contrast, malted wheat contains the necessary enzymes for brewing, allowing the application of 100% of these malts (WM_{100}). To cancel dilution effects, released ferulic acid levels were expressed in milligrams released from 50 g of cereal grains instead of in milligrams per liter.

It was observed that the highest amounts of ferulic acid were released during the first half hour of the mashing process (30 min, 45 °C) (**Figure 3**). At this temperature, the combined action of different enzymes is favorable for arabinoxylan degradation (*39*) and ferulic acid release (*20*, *21*). Furthermore, the results presented in **Figure 3** suggest the occurrence of a ferulic acid releasing enzyme in wheat malt as ferulic acid was released in wort brewed exclusively with this raw material. After 1 h of mashing, ferulic acid levels remained constant, most probably because of enzyme inactivation at higher temperatures. Notably, wort filtration (between 150 and 210 min) even caused a decrease in ferulic acid. It is plausible that ferulic acid is removed from the wort by the formation of new linkages between the phenolic acid and the spent grain filter. Ferulic acid levels in filtered Congress wort ranged from 1.9 to 2.8 mg/L.

On the basis of the higher levels of 4-vinylguaiacol in beer brewed with wheat or wheat malt, it was expected that more of the precursor molecule is released during the beer production process. Nevertheless, the results shown in **Figure 3** do not indicate higher levels of ferulic acid in wort brewed with wheat or wheat malt. On the contrary, more free ferulic acid was solubilized during mashing with 100% barley malt. The level of ferulic acid slightly decreased with increasing level of wheat or wheat malt. This might be caused by a lower level or a lower activity of feruloyl esterases when mashing with wheat or wheat malt. Another plausible explanation can be found in the degradation of arabinoxylans. Several arabinoxylan-degrading enzymes can enhance the release of ferulic acid. However, wheat contains proteins that inhibit xylanase, an important arabinoxylan-degrading enzyme (*39*). The lower ferulic acid content in wort made with wheat or wheat malt might be due to the inhibiting action of these proteins.

Formation of 4-Vinylguaiacol during Boiling. Mashing temperatures (<70 °C) are inadequate for the thermal decarboxylation of ferulic acid as no 4-vinylguaiacol could be detected in unboiled wort. During wort boiling, 4-vinylguaiacol levels increased with increasing boiling times and approximated the threshold value of 0.3 mg/L after 3 h (**Table 3**). Additionally, more 4-vinylguaiacol was formed during boiling of wort made with wheat malt compared to boiling of wort brewed with the same but unmalted wheat. The formation of enzymes and the enzymatic modification of the wheat grain kernels during malting might have contributed to a higher ferulic acid content in wort (2.70 versus 2.20 mg/L). In general, higher amounts of precursor molecules always led to higher 4-vinylguaiacol levels after boiling (data not shown). After an intensive and long boiling regimen, the phenolic flavor compound slightly exceeded

Table 4. Pof Phenotype in Bottom- and Top-Fermenting Brewery Yeast Strains

CMBS no.	yeast type (bottom/top fermenting)	4-vinylguaiacol (mg/L)	odor, –[a] or +[b]	Pof phenotype
1	bottom	0.18	–	Pof⁻
33	bottom	0.09	–	Pof⁻
201	top	15.45	+	Pof⁺
203	top	11.65	+	Pof⁺
210	top	5.44	–	Pof⁺
242	top	0.09	–	Pof⁻
344	top	24.99	+	Pof⁺

[a] Phenolic flavor is undetectable by sensory evaluation. [b] Phenolic flavor is detectable by sensory evaluation.

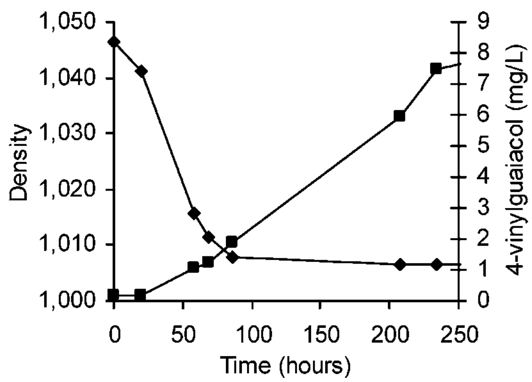

Figure 4. Formation of 4-vinylguaiacol by yeast strain 201 (■) and evolution of wort density (◆) during fermentation of standard 12 °Plato BM_{100} wort supplemented with 50 mg/L ferulic acid.

the threshold value in beer. As this experiment was carried out with a reflux system, little 4-vinylguaiacol evaporated during wort boiling. In a brewery, however, part of the 4-vinylguaiacol is removed together with other volatile compounds. During wort boiling, hops are usually added to acquire bitter and hop aromatic compounds in wort. It was found that the addition of hops caused only a minor increase in the ferulic acid content (<0.1 mg/L). The level of 4-vinylguaiacol was not affected by hop addition.

Selection of Pof⁺ Brewery Yeast Strains. Different strains of *S. cerevisiae* were screened for the Pof⁺ phenotype (**Table 4**). Top-fermenting strains suspected of having an active copy of the *PAD1* (*POF1*) gene were selected from the CMBS yeast library. Two bottom-fermenting strains were included as a negative control. Indeed, according to the results of different researchers (*21*, *23*), bottom-fermenting yeast strains did not reveal the Pof⁺ phenotype. Conversely, decarboxylation of ferulic acid was observed in several top-fermenting brewing yeasts by HPLC as well as by sensory evaluation. Two yeast strains (strains 201 and 344) were selected for further fermentation experiments.

Formation of 4-Vinylguaiacol during Fermentation. In a first fermentation experiment, the development of 4-vinylguaiacol in 12 °Plato wort made with 100% barley malt and supplemented with 50 mg/L of ferulic acid was monitored**.** The evolution of the 4-vinylguaiacol concentration was examined for two selected Pof⁺ yeasts (strains 201 and 344). A moderate formation of 4-vinylguaiacol was observed at the start of the fermentation (**Figure 4**). However, a more remarkable increase in 4-vinylguaiacol formation was observed at the end of fermentation. The levels of the phenolic compound even increased after the depletion of fermentable yeast nutrients. This implies that decarboxylase enzymes retain their activity after fermentation.

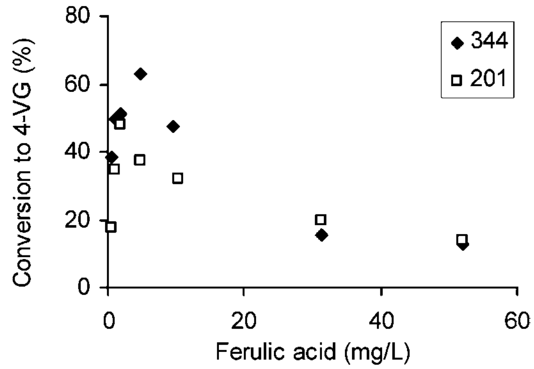

Figure 5. Yield of ferulic acid conversion for two Pof⁺ yeast strains (201 and 344).

The influence of the ferulic acid concentration on the decarboxylation reaction was evaluated in a synthetic medium. High levels of ferulic acid (>50 mg/L) resulted in a lower conversion to 4-vinylguaiacol (**Figure 5**). However, these elevated levels did not completely inhibit the decarboxylation reaction. Furthermore, 4-vinylguaiacol was also formed at very low ferulic acid levels (<0.5 mg/L). It is therefore plausible that the formation of brewing yeast decarboxylase is not limited to a minimal level of 4-vinylguaiacol precursor, in contrast to the decarboxylase of other microorganisms (*40*). The yield of the transformation of ferulic acid to 4-vinylguaiacol also depended on the yeast strain. Compared to yeast strain 201, conversions by strain 344 were higher at low initial ferulic acid concentrations and lower at high initial ferulic acid levels. Maximal yields were obtained for concentrations between 2 and 5 mg/L ferulic acid.

Influence of Wheat and Wheat Malt on Ferulic Acid and 4-Vinylguaiacol Levels during Fermentation. The evolution of ferulic acid levels during fermentation of wort brewed with barley malt, wheat, and wheat malt is presented in **Figure 6**. Wort samples were fermented with yeast strain 344. At the beginning of the fermentation, ferulic acid contents were moderate and slightly higher in Congress wort made with pure barley malt. During fermentation with wort from 100% barley malt, ferulic acid levels remained fairly constant. However, when the $BM_{70}W_{30}$ and $BM_{40}W_{60}$ wort samples were fermented, ferulic acid levels increased remarkably during the first days of the fermentation. The same trends were found for both analyzed wheat cultivars (Genghis and Legat). For fermentations with wheat malt, even more ferulic acid was released during the entire fermentation process.

During the first days of fermentation, pH values decrease because of the formation of organic acids, the uptake of ammonium ions, and the release of protons by yeast cells. However, the strong increase in ferulic acid was not related to acid hydrolysis, as acidification of yeast cell free wort did not lead to an increase in ferulic acid (data not shown). The results indicate the action of a ferulic acid releasing enzyme. It is most unlikely that this activity can be attributed to cereal feruloyl esterase, as the enzyme is denaturated and deactivated during previous wort boiling. Therefore, it is deduced that the activity originates from the Pof⁺ brewing yeast strain. Probably for metabolic reasons, some yeast strains produce both enzymes: feruloyl esterase for the release of phenolic acids and cinnamate decarboxylase for the formation of the corresponding vinyl phenols. The combination of both enzymes was previously found in different yeast species isolated from orange juice (*41*).

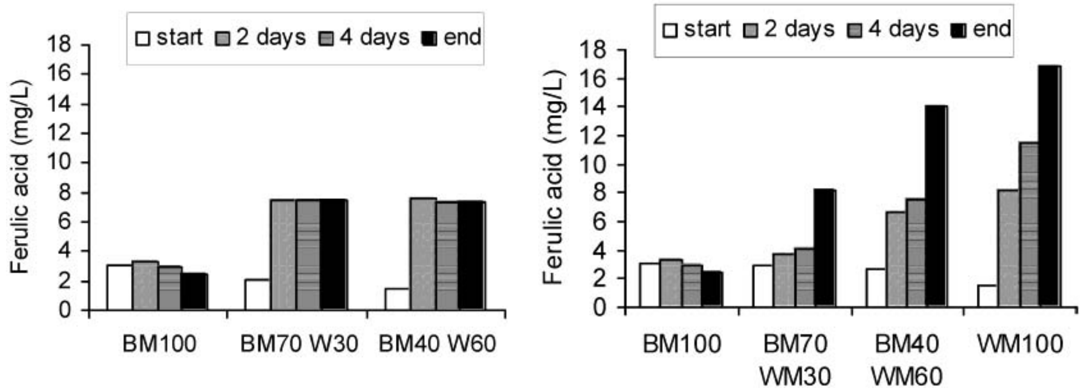

Figure 6. Evolution of ferulic acid levels during fermentation of wort brewed with barley malt (BM) and wheat (W) or wheat malt (WM).

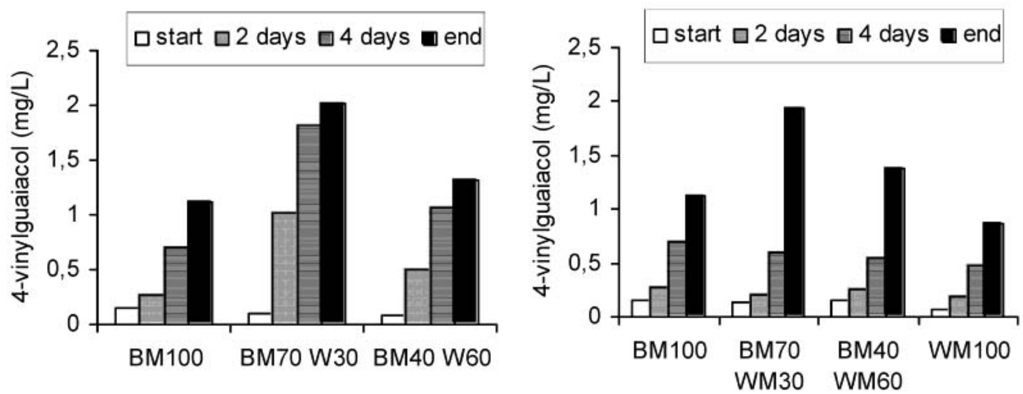

Figure 7. Formation of 4-vinylguaiacol during fermentation wort made with barley malt (BM) and wheat (W) or with barley malt and wheat malt (WM).

However, feruloyl esterase activity in *S. cerevisiae* has not been reported earlier. In contrast to barley malt feruloyl esterase, this enzyme appears to release ferulic acid only from wheat arabinoxylan fragments. Different microorganisms producing enzymes capable of hydrolyzing hydroxycinnamate esters (cinnamoyl ester hydrolase; also termed cinnamoyl esterase, phenolic acid esterase, feruloyl esterase, or *p*-coumaroyl esterase) were reviewed by Williamson et al. (*42*). There is a clear difference in specificity between distinct isolated esterases. Some are almost exclusively active on feruloyl esters or on *p*-coumaroyl esters, whereas others display both activities. The specificity also depends on the linkage between the ferulic acid and the primary sugar (*43*).

The rise of solubilized ferulic acid with increasing levels of wheat or wheat malt might be due to the distribution and structure of arabinoxylans. Ferulic acid appears to be bound only to water-extractable arabinoxylans (*44*). The total amounts of arabinoxylans in barley and wheat are comparable: 2.8−7.1% (w/w) for barley and 3.6−7.1% (w/w) for wheat (*45*). However, the distribution of arabinoxylans in both cereals differs remarkably. Arabinoxylans comprise ∼70% of wheat endosperm cell walls and only 20% of the endosperm cell walls of barley (*46, 47*). In barley, most arabinoxylans can be found in the outer layers as only 22% of total barley arabinoxylan is found in the endosperm (*48*). Malting of barley causes a reduction of the arabinoxylan level (from 3.0 to 1.4%) (*49*). Nevertheless, considerable levels of these pentosans were found in wort (*50*) and beer (*51*), apparently originating from the water-extractable arabinoxylans (*52*). During mashing, accessibility to arabinoxylan-degrading enzymes and release of solubilized arabinoxylan fractions might differ substantially. Endosperm cell walls are unlignified for rapid disintegration during germination. In contrast, lignin- and cellulose-rich cell walls of the outer layers remain mainly intact during germination for physical support of the embryo and the endosperm and for protection of grain kernels against insects and microbial infections. The higher arabinoxylan levels in beer brewed with wheat malt (*51*) could result from the different distribution of arabinoxylans in barley and wheat. Moreover, Dervilly-Pinel et al. (*53*) found that endosperm arabinoxylans from wheat and barley had similar arabinose/xylose ratios. However, the level of ferulic acid per xylose residue and therefore the level of ferulic acid in the endosperm were highest for wheat. Although wheat or wheat malt mashes contain lower levels of free ferulic acid, the level of ferulic acid bound to solubilized arabinoxylan fragments is presumably higher. It is also possible that feruloyl esterase has a lower activity in wort made with barley malt or that ferulic acid bound to barley arabinoxylans is less accessible.

It has been reported that conditions leading to the highest levels of ferulic acid in wort lead to the highest levels of 4-vinylguaiacol in the young beer (*20*). When fermentations were carried out in synthetic medium with added pure ferulic acid, higher concentrations consistently resulted in higher levels of 4-vinylguaiacol (results not shown). However, this was not the case when wheat or wheat malt was used as part of the mash (**Figure 7**). The levels of 4-vinylguaiacol were at a maximum value for 30% wheat or wheat malt. This concentration is frequently applied in the production of wheat and wheat malt beers. Higher levels of ferulic acid do not necessarily give rise to higher levels of 4-vinylguaiacol. It has been observed that volatile phenol synthesis depends on the occurrence of certain inhibitors such as catechins, procyanidins, anthocyanins, and tannins (*28*). Our results also show that yeast decarboxylase is clearly affected by the presence of certain wheat components as yet undetermined.

Ferulic Acid and 4-Vinylguaiacol in Commercial Beers. The levels of the phenolic compound 4-vinylguaiacol and its precursor ferulic acid were analyzed in several brands of five

Table 5. Ferulic Acid (FA) and 4-Vinylguaiacol (4-VG) Levels in Commercial Pilsner Type (P), Ale (A), Strong Blond (SB), Wheat (W), and Wheat Malt (WM) Beers

beer	FA (mg/L)	4-VG (mg/L)	beer	FA (mg/L)	4-VG (mg/L)
P1	2.07	ND	SB1	5.92	0.53
P2	6.44	0.28	SB2	1.39	1.48
A1	6.60	ND	SB3	1.98	1.59
A2	3.28	ND	SB4	1.20	1.71
A3	4.84	0.13	SB5	2.11	1.84
A4	7.64	0.16	SB6	1.58	2.23
A5	1.65	0.22	SB7	9.41	3.98
A6	2.29	0.24	SB8	12.15	2.43
A7	4.43	0.26	SB9	14.19	4.10
W1	8.19	0.16	WM1 (white)	3.92	0.15
W2	4.45	1.09	WM1 (dark)	3.74	0.24
W3	2.66	1.16	WM2 (white)	11.61	1.14
W4	2.75	1.47	WM2 (dark)	10.80	0.96
W5	4.04	1.42	WM3 (white)	4.31	2.24
W6	3.29	1.95	WM3 (dark)	3.48	1.85
W7	3.86	1.86	WM4 (white)	5.21	2.42
W8	8.24	2.23	WM4 (dark)	6.07	1.99

different types of beer: pilsner, ale, strong blond, wheat, and Weizen (**Table 5**). For the Weizen beers, both a white and a dark variety were assessed. In the analyzed beers, ferulic acid levels were high compared to the levels in Irish brands but comparable with levels in American and other European beers (*54*, *55*). The higher levels of ferulic acid possibly arise from the use of well-modified malts, initially low mashing temperatures, or wheat or wheat malt. In pilsner and ale beers, little or no 4-vinylguiaicol could be detected by HPLC analysis. Despite rather high levels of ferulic acid in some of the analyzed beers, without the use of a Pof$^+$ yeast, 4-vinylguaiacol is formed only at moderate levels during the brewing process. On the contrary, strong blond beers, wheat beers, and Weizen beers are frequently fermented with Pof$^+$ yeasts. For these styles, low 4-vinylguaiacol levels are quite exceptional. Nevertheless, it was difficult to predict which beers were brewed with wheat or wheat malt from the ferulic acid level. The application of wheat or wheat malt does not always result in ferulic acid rich beers. It is clear that for commercial beers, ferulic acid levels depend on many factors. Not only malt modification, type of raw material, and initial mash temperature and pH but also technique and duration of wort filtration or the application of phenolic-removing beer filter aids (*56*) such as polyvinylpyrrolidone (PVP) and polyvinylpolypyrrolidone (PVPP) can influence the level of ferulic acid in beer. In this study it is proposed that yeast esterase activity may contribute to the release of ferulic acid during fermentation of wort brewed with wheat or wheat malt. The level of 4-vinylguaiacol in beer is determined by thermal and, for the most part, enzymatic decarboxylation. When fermentation is made with a Pof$^+$ yeast, 4-vinylguaiacol formation depends on the used yeast strain, the fermentation conditions and parameters, and the ferulic acid content. In addition, development of 4-vinylguaiacol might be inhibited by certain wheat components.

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