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A N I N T E R N A T I O N A L J O U R N A L

Determination of 4-vinylgaiacol and 4-vinylphenol in Top-fermented Wheat Beers by Isocratic High Performance Liquid Chromatography with Ultraviolet Detector

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ABSTRACT

This work aimed at determining 4-vinylguaiacol and 4-vinylphenol in the top-fermented wheat beers using different wavelength and the mobile phase for HPLC. Best results for isocratic elution were obtained at 260 nm and the mobile phase comprising methanol/ultrapure water/phosphoric acid (400/590/10, V/V). Under these conditions, the retention time of 4-vinylguaiacol and 4-vinylphenol was 25 and 27min, respectively.

Key words: 4-vinylguaiacol, 4-vinylphenol, Top-fermented wheat beers, HPLC, phenolic off-flavor

INTRODUCTION

Despite being considered undesirable and cataloged as a “phenolic off-flavor” (POF) in bottom-fermented beers (Thurston et al. 1981; Thurston et al. 1986), 4-vinylguaiacol (4VG) and 4-vinylphenol (4VP) are well-known aroma compounds found in top-fermented beers, including Belgian white beers (brewed with unmalted wheat), German Weizen beers and Rauch beers (brewed with malted wheat) (Coghe et al. 2004; Vanbeneden et al. 2006; Vanbeneden et al. 2008). Both the 4VG and 4VP have a lower sensory threshold value, which are reported to be 0.3 and 0.2 mg/L, respectively (Thurston et al. 1981; BDAS Online. 2006; Vanbeneden et al. 2006). Even at trace levels, they can also produce a significant medicinal, phenolic, clove-like, smoky, or BBQ flavor in beers (Thurston et al. 1981; Thurston et al. 1986; McMurrough et al. 1996; Coghe et al. 2004; Vanbeneden et al. 2008). Hence, they are of vital importance for the overall flavor perception in these top-fermented beers.

The 4VG and 4VP have the similar structure (Fig. 1), (Vanbeneden et al. 2006; Vanbeneden et al. 2008). Their molecular weights are 150.18 and 120.15, respectively. The universal method to determine the 4VG and 4VP is by High Performance Liquid Chromatography (HPLC) with gradient pump (McMurrough et al. 1996, Shinohara et al. 2000; Vanbeneden et al. 2008). Nevertheless, there are also different analysis methods for different detectors. For example, for fluorescence monitor, mobile phase is $H_2O/CH_3OH/H_3PO_4$=540:450:10 and excitation and emission wavelength is 259 nm and 341 nm, respectively (McMurrough et al. 1996); for U.V. detector, mobile phase is $H_2O/CH_3CN/H_3PO_4$=599:400:1 and wavelength is 230 nm (Shinohara et al. 2000); for electrochemical detector, mobile phase is $H_2O/CH_3OH/H_3PO_4$=745:245:10, and the working electrode is Ag/AgCl, +1200 mV, 100 nA

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(Vanbeneden et al. 2006; Vanbeneden et al. 2008), etc.

This work aimed to study the determination of 4VG and 4VP in top-fermented wheat beers by isocratic HPLC with ultraviolet detector.

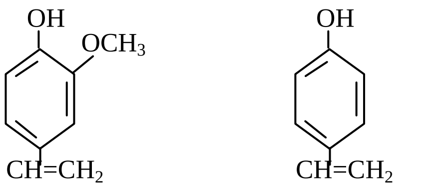

Figure 1 - The chemical structure of 4VG (left) and 4VP (right).

EXPERIMENTAL

Reagents and materials

HPLC grade 4VG (≥98%, 2-methoxy-4-vinylphenol) and 4VP (10% in propylene glycol) were purchased from SAFC (UK) and Alfa Aesar (Tianjin, China), respectively. HPLC grade methanol and acetonitrile were obtained from Tianjin Siyou Chemical Reagent Co, Ltd (Tianjin, China) and Jinan Institute of Chemical Engineering (Jinan, Shandong, China), respectively. Phosphoric acid (85%) was analytical grade. The ultrapure water used in this study was deionized and filtered by a Milli-Q Plus water purifying system (Bedford, MA, USA). Scarlett barley malt was procured from Zhongliang Malt Division (Dalian, China). Wheat malt was obtained from Weyermann (Bamberg, Germany). Hops were supplied from Barth-Haas Group (Beijing, China), which contained 4.9% α-acids (Analytica-EBC) (Cui et al. 2010).

HPLC system

LC-10AT HPLC (Shimadzu) and Rheodyne 7725i manual sampler were used. Absorption spectra were recorded with a UV spectrophotometer, model SPD-10Avp. Isocratic separation was performed on a reversed-phase C_{18} column (4.6 mm×150 mm, 5 μm particle size, Diamonsil). The measurements were carried out at room temperature. The flow rate was at 1 mL/min. The injection volume was 20 μL.

Preparation of 4VG and 4VP standard solutions

According to the density of 4VG and 4VP as 1.10 and 1.04 mg/L, respectively, the standard solution of 4VG and 4VP were prepared as mentioned in Table 1.

Table 1 - Preparation of 4VG and 4VP standard solution.

	Concentrations of standard substance	Preparation methods
4VG	15 μL×1.10 g/mL ÷ 250 mL = 66 mg/L	15 μL standard substance+149.985 mL mobile phase
4VP	300 μL×1.04 g/mL×10% ÷ 50 mL = 624 mg/L	300 μL standard substance+49.700 mL mobile phase

Preparation of samples

The top-fermented wheat beers were fermented in the pilot-scale brewery (20 hL) in Shandong Polytechnic University. Brewing process was carried out as follows. barley malt (102 kg), wheat malt (68 kg), brewing water (5.95 hL), mashing procedure: 44°C (20 min), 52°C (40 min), 65°C (70 min), 72°C (15 min), 78°C (10 min), wort lautering by lauter tun, boiling for 90 min in wort kettle, wort clarification in whirlpool, original gravity is 11°P, fermentation temperature 20°C (5 days), lagering period 7 days at 0°C (Cui et al. 2010).

Mobile phases and samples were filtered through 0.45 μm pore PVDF membrane filters and then decarbonated by sonication. All the samples were protected from light during operation to minimize the photo-isomerisation reaction.

The determination of the optimized absorption wavelength

Wavelength ranges were set between 190-300 nm on the ultraviolet spectrophotometer to confirm the optimal absorption wavelength.

The determination of the optimal mobile phase

The 4VG and 4VP standard solutions were mixed at the rate of 1:1. The mobile phases consisted of acetonitrile/ultrapure water/phosphate and methanol/ultrapure water/phosphoric acid as shown in Table 2.

Table 2- Different mobile phase used in the study.

No.	Mobile phase
A	Acetonitrile/ultrapure water/phosphoric acid =300/690/10
B	Acetonitrile/ultrapure water/phosphoric acid =200/790/10
C	Methanol/ultrapure water/phosphoric acid =400/590/10
D	Methanol/ultrapure water/phosphoric acid =600/390/10

The determination of 4VG and 4VP concentrations in top-fermented wheat beers
The top-fermented wheat beers were injected into HPLC under the conditions of optimized absorption wavelength and mobile phase in this study so as to determine the levels of 4VG and 4VP.

RESULTS AND DISCUSSION

The determination of the optimum absorption wavelength
The wavelength (190~300 nm) scanning maps of 4VG and 4VP are shown in Figure 2, respectively. There was a relatively better absorption peak at 260 nm for both the 4VG and 4VP. Furthermore, according to the proper property of acetonitrile and methanol, the absorption values of them were less than 0.005 and 0.01 at 260 nm, respectively. Hence, it was an appropriate choice to set the wavelength at 260 nm to separate the 4VG and 4VP entirely.

The determination of the optimal mobile phase
The 4VG and 4VP standard solutions were mixed at the rate of 1:1 and their standard solutions with different mobile phases shown in Table 2 were injected into HPLC system. The corresponding spectra are shown in Figure 3.
Figure 3C showed that the peaks of 4VG and 4VP could be separated completely and the analysis time was the shortest, so the optimal mobile phase was methanol/ultrapure water/phosphoric acid (400/590/10, V/V) and the analysis time of 4VP and 4VG was about 25 and 27 min, respectively.

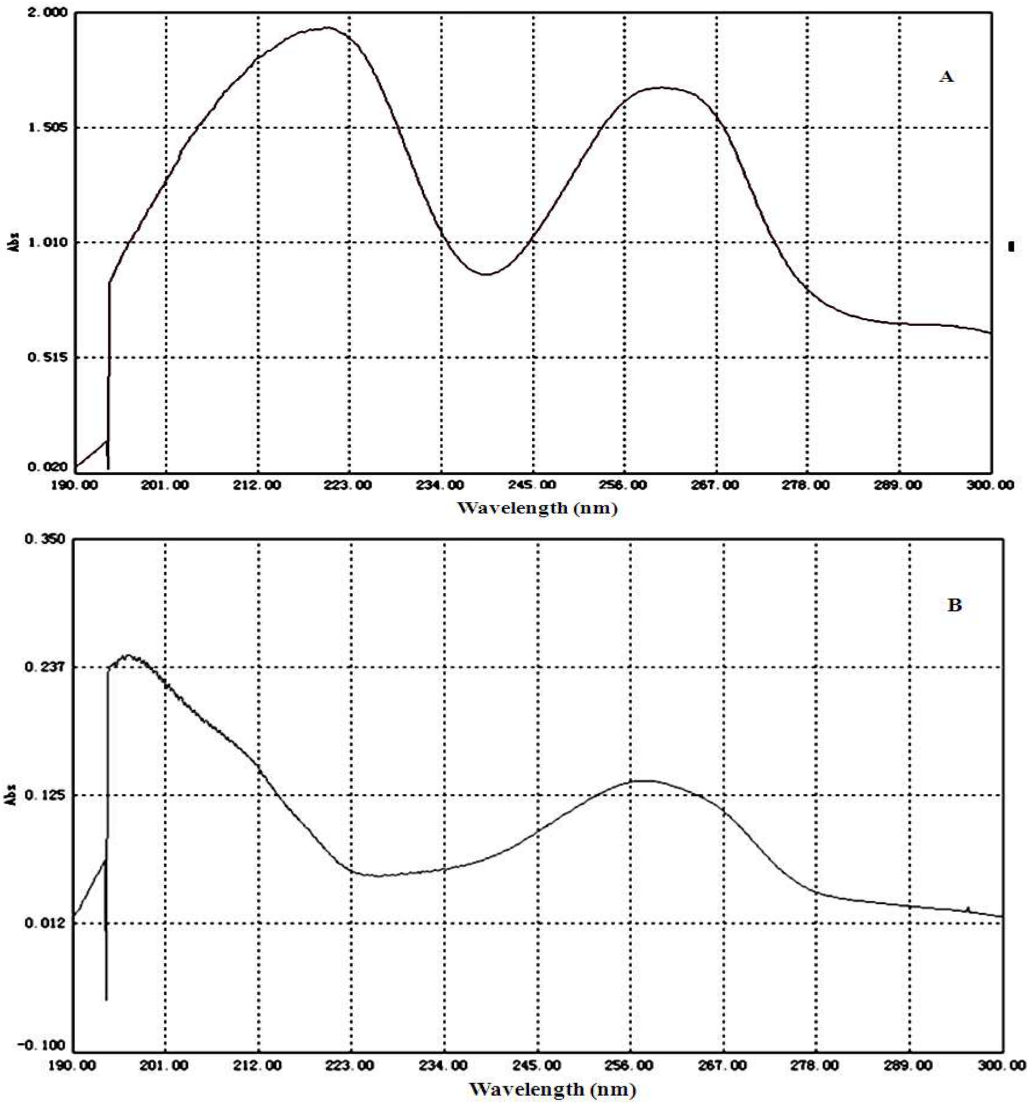

Figure 2 - The wavelength scanning maps of 4VG (A) and 4VP (B).

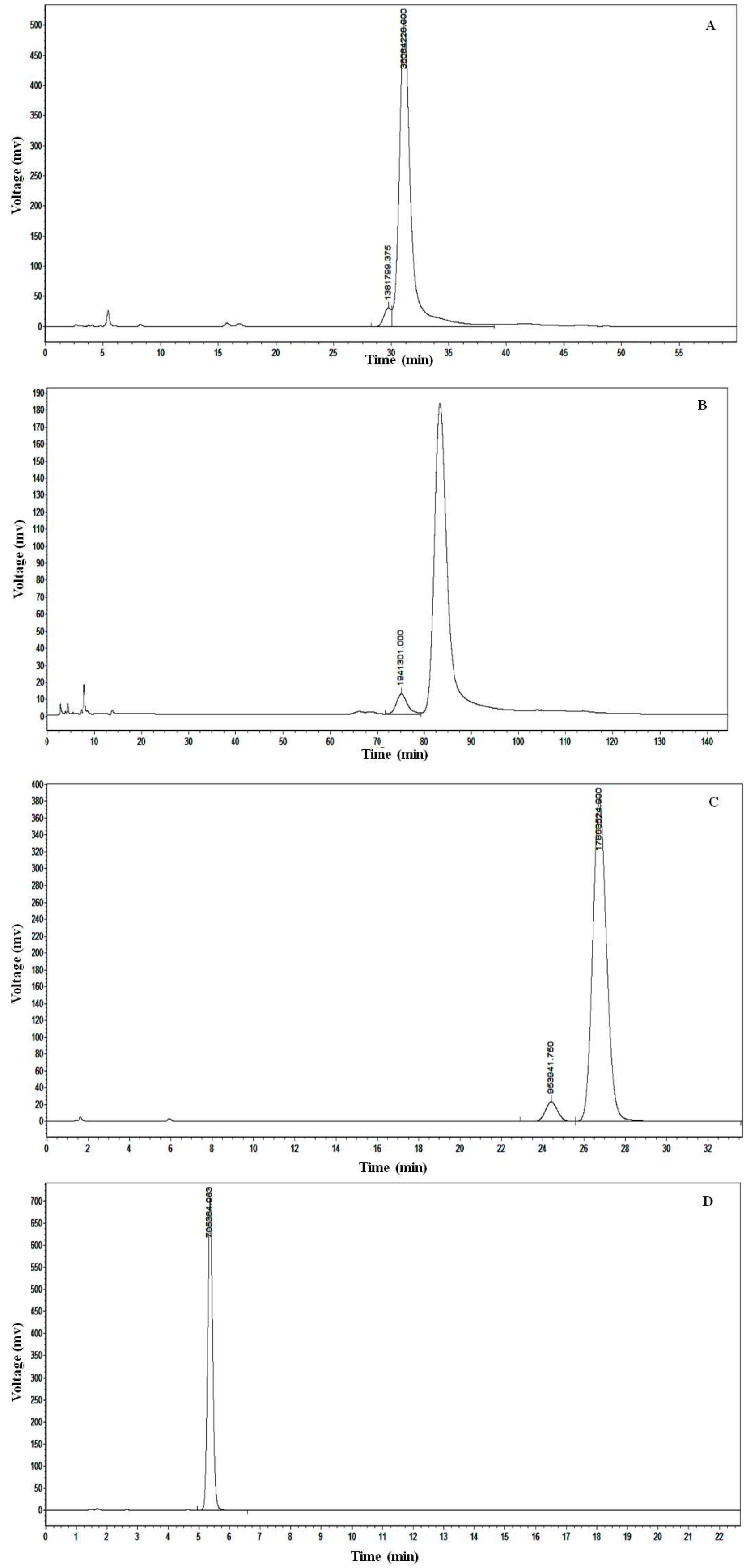

Figure 3 - Standard solution spectra eluted with different mobile phase (No. A-D in Table 2).

The drawing of 4VG and 4VP standard curves

The five concentration gradients of 4VG or 4VP were prepared and injected into HPLC according to standard operations. Peak areas were obtained as shown in Table 3. Consequently, standard curves of 4VG and 4VP were drawn (Fig. 4).

Table 3 - Concentrations and peak areas of 4VG and 4VP.

	1	2	3	4	5
4VG concentration	1.110	2.220	2.960	4.440	5.550
4VG peak area	62.428	90.100	12.8940	19.7724	22.7081
4VP concentration	0.416	0.832	1.248	1.664	2.080
4VP peak area	16.407	28.802	46.814	63.817	78.743

Determination of 4VG and 4VP concentrations in top-fermented wheat beers

The top-fermented wheat beers were injected into HPLC under the conditions of optimized absorption wavelength and mobile phase. The, spectrum of top-fermented wheat beers and close-up spectrum of 4VG and 4VP are shown in Figures 5 and 6, respectively.

The 4VG and 4VP peaks in Figure 6 were substituted in the standard curve equations of 4VG and 4VP in Figure 4. After calculation, the contents of 4VG and 4VP in the top-fermented wheat beers found were 2.6133 and 1.0095 mg/L, respectively, which was similar to the findings of Wackerbauer et al. (1982) and Schwarz et al. (2012).

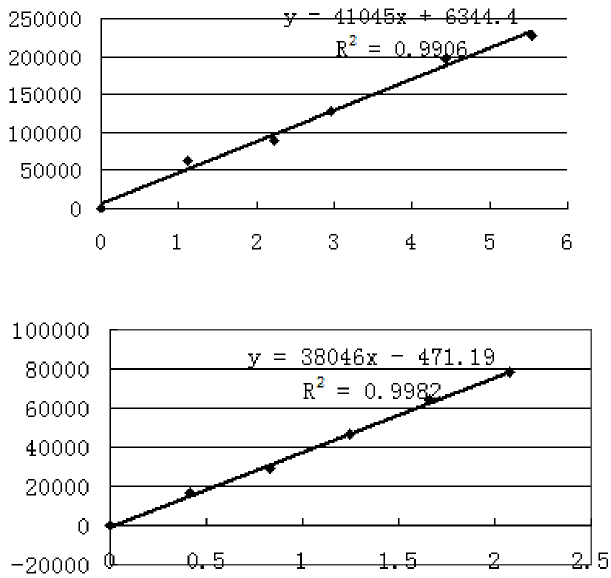

Figure 4 - Standard curves of 4VG (left) and 4VP (right).

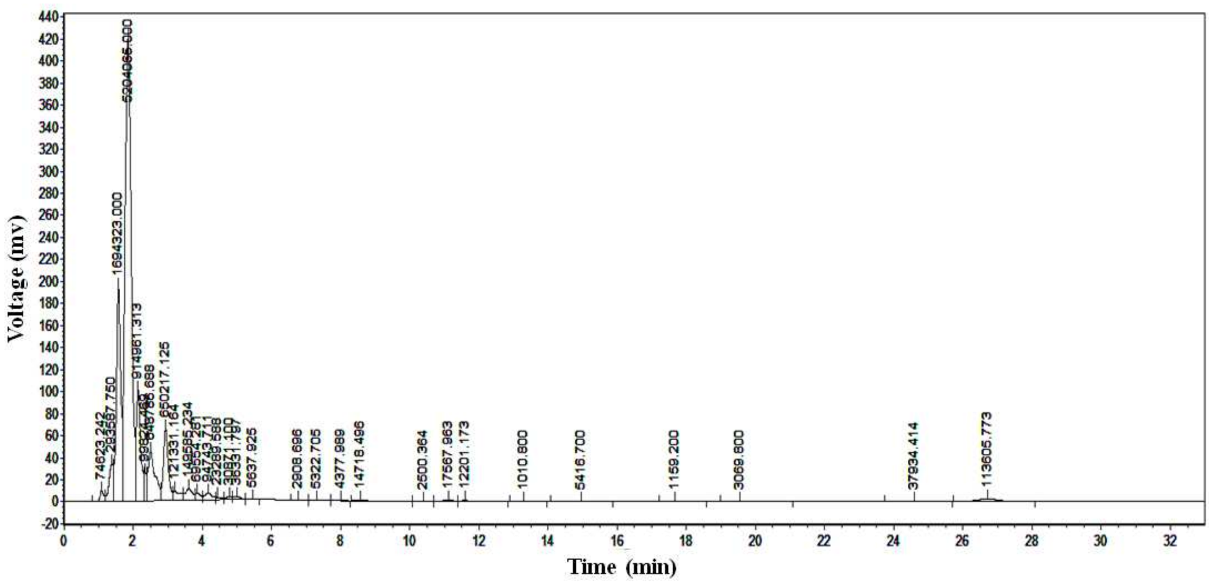

Figure 5 - Spectrum of top-fermented wheat beers.

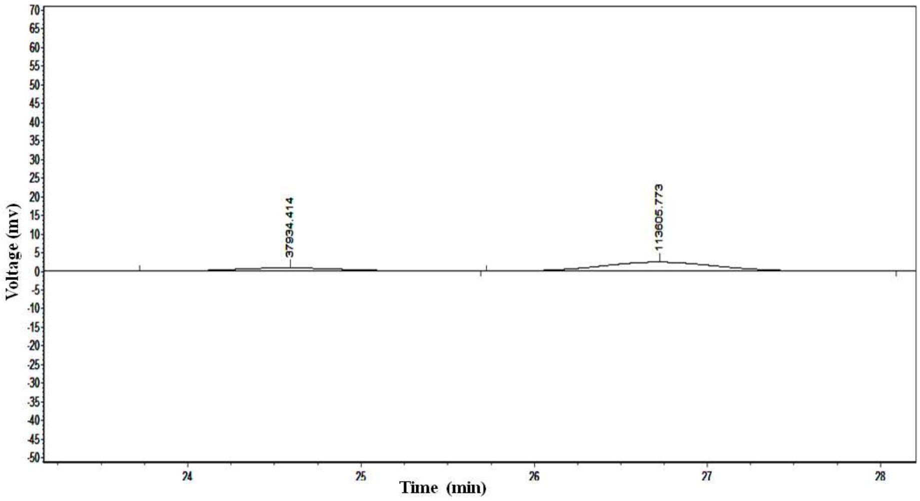

Figure 6 - Close-up spectrum of 4VG (right) and 4VP (left).

CONCLUSION

In summary, under the existing conditions (LC-10AT, 7725i manual sampler, SPD-10Avp UV spectrophotometer, reversed-phase C_{18} column), the optimum HPLC conditions were wavelength at 260 nm, the mobile phase methanol/ultrapure water/phosphoric acid (400/590/10, V/V), flow rate 1.0 mL/min and injection volume 20 μL. This resulted the analysis time of the 4VP and 4VG as 25 and 27 min, respectively.

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REFERENCES

BDAS Online [homepage on the internet]. Kentucky USA: Brewing and Distilling Analytical Services, LLC, c2003-2013 [cited 2006 Oct. 10]. Available from: http://www.alcbevtesting.com/testing-facilities/flavor-corner.

Coghe S, Benoot K., Delvaux F, Vanderhaegen B, Delvaux FR. Ferulic Acid Release and 4-Vinylguaiacol Formation during Brewing and Fermentation: Indications for feruloyl esterase activity in *Saccharomyces cerevisiae*. *J Agric Food Chem*. 2004; 52(3): 602-608.

Cui YQ, Cao XH, Li SS, Thamm L, Zhou GT. Enhancing the concentration of 4-vinylguaiacol in top-fermented beers-a review. *J Am Soc Brew Chem*. 2010; 68(2):77-82.

McMurrough I, Madigan D, Donnelly D, Hurley J, Doyle AM, Hennigan G, et al. Control of ferulic acid and 4-vinyl guaiacol in brewing. *J Inst Brew*. 1996; 102:327-332.

Schwarz KJ, Stübner R, Methner FJ. Formation of styrene dependent on fermentation management during wheat beer production. *Food Chem*. 2012; 134 (4):2121-2125.

Shinohara T, Kubodera S, Yanagida F. Distribution of phenolic yeasts and production of phenolic off-flavours in wine fermentation. *J Biosci Bioeng*. 2000; 90 (1):90-97.

Thurston PA. The phenolic off-fllavor test: a method for confirming the presence of wild yeasts. *J Inst Brew*. 1986; 92: 9-10.

Thurston PA, Tubb RS. Screening yeast strains for their ability to produce phenolic off-flavours: A simple method for determining phenols in wort and beer. *J Inst Brew*. 1981; 87: 177-179.

Vanbeneden N, Delvaux F, Delvaux FR. Determination of hydroxycinnamic acids and volatile phenols in wort and beer by isocratic high-performance liquid chromatography using electrochemical detection. *J Chromatogr A*. 2006, 1136 (2):237-242.

Vanbeneden N, Gils F, Delvaux F, Delvaux FR. Formation of 4-vinyl and 4-ethyl derivatives from hydroxycinnamic acids: Occurrence of volatile phenolic flavour compounds in beer and distribution of Pad1-activity among brewing yeasts. *Food Chem*. 2008; 107 (1):221-230.

Wackerbauer K, Kramer P. Bavarian wheat beer - an alternative production and composition. *Brauwelt*. 1982; 122 (18):758-762.

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